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FORBIDDEN LINES IN ASTROPHYSICAL SOURCES

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The successful interpretation, by Bowen,[1] of the strongest unidentified nebular lines in terms of transitions from metastable levels, was concerned with the spectra of N II, O II and O III. Later identifications of forbidden lines of O I and S II in nebulae were made by Bowen,[2,3] forbidden Fe II lines were found by Merrill[4] in η Carinae, and the green auroral line was noted by McLennan[5] as arising from a metastable transition in the spectrum of O I.

Bowen's discovery was a logical sequel to the recognition of the spectroscopic importance of low nebular densities.[6] His first paper discussed all the atoms, of likely occurrence and moderate ionization, whose spectra were at the time analyzed well enough for their metastable states to be located. His identifications were made on the basis of accurately known frequency-differences for related pairs of lines (such as the "nebular lines" N_1 and N_2), and on less accurately known wave-lengths determined from series formulae and from assumed quantitative similarities between related spectra. All the identifications thus made for N II, O II and O III were substantiated by later astrophysical work, and aided in the further laboratory analysis of some of the spectra,[7] making possible the discovery of intersystem combinations. The forbidden lines that have hitherto been recognized astrophysically are tabulated by Miss Moore.[8]

The recent advances in the analysis of spectra, as summarized in Miss Moore's tables, and in Bacher and Goudsmit's compilation,[9] have made possible a more complete survey of the metastable states of the atoms from hydrogen to argon. The present note summarizes the results of this survey, which has led to the certain or probable identification of a number of new forbidden lines in astrophysical sources.

Among the neutral atoms from hydrogen to argon there are three types, whose electronic configurations of the ground states give rise to metastable levels. These are shown schematically in figure 1; transitions of types *a*, *b* and *c* will hereafter be called "nebular," "auroral" and "trans-

auroral" (transition "*a*" produces the "nebular lines" N_1 and N_2 of O III, and transition "*b*" gives the "auroral" line of O I). It is seen that the figures to the left and right, corresponding to configurations p^2 and p^4, are similar except that the lowest level is inverted in the latter. Therefore, if the usual intensity relations hold, we should expect the nebular component of shorter wave-length to be the stronger for spectra arising from p^4, and the weaker for spectra arising from p^2. This intensity relationship, substantiated for Bowen's lines of O I, O III and N II, is an important additional criterion for identification.

In identifying the nebular pair of O I, Bowen noted that the corresponding auroral line is absent from the nebulae, while the nebular lines of O I are absent from, or inconspicuous in, the aurora,[10] and he discussed the difference of conditions in the two sources. The same phenomenon is shown by the forbidden lines of O III, for in the spectra of nebulae the nebular transitions are, with few exceptions, stronger than the auroral transitions. It is, however, well known[11] that the auroral line of O III is the first line of that atom to appear in the developing spectrum of a nova, and that for some time after the appearance of the nebular lines the auroral line is very much the stronger. In the course of evaluating this observation, which is discussed in a paper to be published shortly, it transpired that the novae provide a rich source for the study of forbidden transitions, since they effectively run through a series of stages corresponding to a series of nebulae arranged in order of increasing excitation, and probably decreasing density.

Recent studies of the Wolf-Rayet stars[12] make it appear that they are similar to compact planetary nebulae of very high excitation, though they show no forbidden lines, presumably because of too high a density and too intense radiation. Roughly speaking, Wolf-Rayet stars, novae and nebulae have the same excitation: Wolf-Rayet stars show no forbidden lines; the novae (especially in their early stages) show most conspicuously the auroral lines, and the nebulae show most strongly the nebular transitions.

The present state of our information as to the occurrence of forbidden lines in the spectra of lighter elements is summarized in table 1. Two types of metastable states may be considered, those arising from the same electronic configuration as the ground state and those arising from other electronic configurations. Among the lighter elements forbidden lines arising from this latter type of metastable state would be too far in the ultra-violet for observation, and consideration is therefore limited to those atoms or ions, the electronic configuration of whose ground state also gives rise to metastable levels. These are the configurations p^2, p^3 and p^4. Isoelectronic sequences of such configurations are arranged vertically, and three columns, headed *a*, *b* and *c*, refer, respectively, to nebular, auroral

and transauroral transitions. The letters *P* or *A* indicate whether the corresponding forbidden line is present or absent; "*r*" and "*v*" show that forbidden transitions should exist, but are too far respectively to the red and violet to be identified in astrophysical sources, at least with our present observational data. An asterisk before the atomic symbol shows that the normal line of the corresponding atom is present in stellar spectra.

TABLE 1

PREDICTED AND OBSERVED FORBIDDEN TRANSITIONS

p^2	*a*	*b*	*c*	p^3	*a*	*b*	*c*	p^4	*a*	*b*	*c*
C I	*r*	*r*	*P*	N I	*p*	*r*	*A*?	*O I	*P*	*P*	*v*
*N II	*P*	*P*	*v*	*O II	*P*	*P*	*v*	F II	?	?	*v*
*O III	*P*	*P*	*v*	F III	?	*P*	*v*	Ne III	*P*	*P*	*v*
F IV	?	?	*v*	Ne IV	*v*	*P*	*v*	Na IV	?	*v*	*v*
Ne V	*v*	?	*v*	Na V	*v*	?	*v*				
*Si I	*r*	*r*	*P*?	P I	*r*	?	?	S I	*r*	*r*	?
*P II	*r*	?	?	*S II	*P*	*r*	*P*	Cl II	*r*?		
*S III[13]	*r*	*P*?	?	Cl III	*P*?	*r*	?	A III	?	?	*v*
Cl IV	?	?	*v*	A IV	?	*r*	*v*?	K IV			

The bases for the positive entries in table 1 will now be briefly summarized. The criteria for the identification of a forbidden line in the spectrum of a nebula or nova are as follows, roughly in order of importance.

1. For nebular and transauroral pairs, the wave-number separations must be equal to the separation of the corresponding levels of the ground state. This is usually the test that can be most accurately applied, and is the one most useful in a search for a likely identification. For example, the nebular pair now ascribed to Ne III was first suspected to belong to that atom because the same wave-number separation had been found in the laboratory.[14] Because the wave-number separations are usually accurately known, the identification of nebular pairs is easier and surer than the identification of single lines.

2. The relative intensities of the lines forming a nebular pair are determined by the weights of the corresponding states, and should remain the same wherever the pair occurs. Data on this point will be found in table 3.

3. An approximate wave-length may be determined by using a Moseley extrapolation along an isoelectronic sequence, by assuming constancy of quantum defect in certain cases, or by calculating the series limits of the next lower stage of ionization. All of these methods are subject to uncertainties, and therefore, unless the relative position of the metastable levels is also fixed in other ways (as when intersystem combinations are known), such estimates can be used only roughly to guess in what part of the spectrum the line considered may be found. In table 2 the methods of predicting the wave-lengths of forbidden lines are indicated by symbols.

4. When a forbidden line is observed in a nova spectrum, the identification cannot be considered satisfactory unless the line appears at about the same time as other forbidden lines of similar type (auroral, nebular or transauroral) associated with similar energy of excitation.

5. No forbidden line should appear in absorption. If an absorption line at the same wave-length has been recorded, it must be satisfactorily identified as coming from an atom that would not be expected to give a bright line under nebular conditions.

6. In general, an emission line recorded in stellar spectra is unlikely to arise from a forbidden transition, though, as in (5), a fortuitous coincidence may be responsible. Exceptions are noted for peculiar objects such as CI Cygni, RW Hydrae and AX Persei,[15] which combine stellar and nebular characteristics.

The identification of the nebular pair of Ne III requires special discussion. Since no intersystem lines have yet been identified in this spectrum the position of $^1D_2$ relative to $^3P_{210}$ can be estimated only from the series limits of Ne II. Russell, Compton and Boyce[16] identified the lowest terms of series converging to the respective limits $^1D_2$ and $^1S_0$ of the next higher stage of ionization. They gave tentative values for these limits, based on the assumption that the terms have the same quantum defect as the most nearly corresponding term of the series that converges to the $^3P_{210}$ limit. Such an approximation gives the position of the nebular pair for Ne III as 4483.1 and 4594.3. The more complete analysis of O II by Russell[7] shows, however, that this constancy of quantum defect is not exact, a shift of 0.034 in its value being indicated. If the value of the quantum defect for Ne III is shifted by the same amount, the calculated positions of the forbidden lines of Ne III agree remarkably well with the observed positions of the strong nebular pair. In view of the other evidence (the exactitude of the wave-number separation, the intensity ratio of the two lines, and their position in the nova excitation sequence), this shift seems entirely justified. The identification of the auroral line of Ne III with the observed line at 3342 must still be regarded as provisional. The nebular wave-lengths in this general region are somewhat uncertain, since they were derived by extrapolation upon slitless spectrograms.

The lines are strong in practically all the nebulae and absent from only two in Wright's list: N.G.C. 40 and I.C. 418—objects whose spectra are indicative of low excitation. Waiving the question of relative atomic abundance, the persistence of the nebular lines of O III in these spectra suggests that the atom in question requires still higher excitation. On the other hand, 3869 and 3968 are very intense in I.C. 4997, whose spectrum shows 3727 O II strongly and the nebular lines of O III only weakly. This nebula, however, is peculiar in having the auroral line of O III as the

most intense in its whole observed spectrum; behavior of this sort should warn us that the intensity of the nebular lines of O III is not an infallible index to the degree of excitation of a particular nebula. The estimate of Bowen and Menzel,[17] that the atom producing 3869 and 3968 has an ionization potential intermediate between those of O II and O III, was subject to considerable uncertainty.

A final and apparently conclusive argument in favor of assigning the lines to Ne III is furnished by their behavior in Nova Persei, of whose spectrum the two lines became by far the strongest feature, confirming Wright's conclusion[18] that they are a related pair. The prominence of Ne III at once suggested that Ne IV (predicted position 4712) should also be present; in the later stages of Nova Persei a very intense line (also recorded in nebulae) appears at about 4725. Although the difference between the observed and calculated positions is considerable, the astrophysical evidence favors the assignment of 4725 to the atom of Ne IV. Thus the two strongest lines hitherto unidentified in the nebular spectrum are now recognized as due to neon, and a new element is added to the list of those known astrophysically.

TABLE 2

LIST OF OBSERVABLE FORBIDDEN LINES

ATOM AND I.P.	TRANSITION	λ_{CALC}	λ_{OBS} NEBULA	λ_{OBS} NOVA	OTHER SOURCES
C I	$^3P_1 - {}^1S_0$	4621.5	No	4618 Nova Gem II[1]	
11.2	transauroral	*		4625 Nova Aur[c]	
				4625 Nova Lac[2]	
				4625 Nova Gem I[2]	
N I	$^4S^o_{1\frac{1}{2}} - {}^2D^o$	5210	No	5210.8 Nova Pic[3]	
14.5	nebular	‡			
	$^4S^o_{1\frac{1}{2}} - {}^2P^o$	3470	No	No	
	transauroral	‡			
N II	$^1D_2 - {}^1S_0$	5754.8	5755.2 NGC 6572[4]	5754 Nova Aql[5]	
29.5		*	5754.4 NGC 7027[4]	5755 Nova Gem[6]	
	auroral		5755.2 +30° 3639[4]	5752.7 Nova Pic[3]	
				5754 Nova Lac[3]	
	$^3P_1 - {}^1D_2$	6547.0 6548.3	NGC 6572[4]	....Nova Aql[7]	
		* § 6583.2			
	$^3P_2 - {}^1D_2$	6583.3 6548	NGC 7027[4]		
		* § 6583			
	nebular	6548.3	+30° 3639[4]		
		6583.9			
O I	$^1D_2 - {}^1S_0$	5577.3	No	5577 Nova Gem[6]	5577 Aurora[8]
13.56	auroral	*		5577 Nova Aql[5]	
	$^3P_2 - {}^1D_2$	6300.3 6301.4	NGC 6572[4]	6302 Nova Gem[6]	
		* § 6367			
	$^3P_1 - {}^1D_2$	6363.7 6303.6	+30° 3639[4]	6300	Nova Aql[5]
	nebular	* § 6300.1	NGC 7027[4]	6371	
		6363			

O II 34.9	$^2D^o_{2\frac{1}{2}} - {}^2P^o_{1\frac{1}{2}}$	7321 * §	7319.9	NGC 7027[9]	red			
				NGC 6572[9]				
	$^2D^o_{2\frac{1}{2}} - {}^2P^o_{\frac{1}{2}}$	7324 * §						
	$^2D^o_{1\frac{1}{2}} - {}^2P^o_{1\frac{1}{2}}$	7332 * §	7330.4	NGC 7027[9]				
				NGC 6572[9]				
	$^2D^o_{1\frac{1}{2}} - {}^2P^o_{\frac{1}{2}}$ auroral	7334 * §						
	$^4S^o_{1\frac{1}{2}} - {}^2D^o_{1\frac{1}{2}}$	3725.1 * §	3726.4 3729.1	Orion Neb.		Nova Aql[10]		
						Nova Gem[6,11]		
	$^4S^o_{1\frac{1}{2}} - {}^2D_{2\frac{1}{2}}$	3727.8 * §	3726.22 3728.9	NGC 6572				
	nebular		3726.22 3728.94	IC 418				
O III 54.9	$^1D_2 - {}^1S_0$	4363.1 *	4363.31 4363.29	NGC 6543 NGC 6572	4363.1 4363.4	Nova Pic[3] Nova Gem[6]	4363.24	Z And[15]
	auroral							
	$^3P_1 - {}^1D_2$	4958.9 * §	4959.09 5007.0	NGC 6543	4959.6 5007.9	Nova Pic[3]	4958.8 5006.9	Z And[15]
	$^3P_2 - {}^1D_2$	5007.1 * §	4959.15 5007.04	NGC 6572	4960 5007	Nova Gem[6]		
	nebular							
F II 34.6	$^1D_2 - {}^1S_0$	4150 †		No	4178.9	Nova Pic[3,12]		
	auroral							
	$^2P_2 - {}^1D_2$	4808 † ‖		No	5172.7	Nova Pic[3,13]		
	$^2P_1 - {}^1D_1$	4889 † ‖		No	5271.4	Nova Pic[3,13]		
	nebular							
F III 60	$^2D^o - {}^2P^o$	5725.1 *		No ?	5723.3	Nova Pic[3]		
	auroral					Nova Aql[14]		
F IV 84	$^3P_1 - {}^1D_2$	3887.2 † §		No		No		
	$^3P_2 - {}^1D_2$	3914.9 † §		No		No		
	nebular							
Ne III 63	$^1D_2 - {}^1S_0$	3338 *	3342 3342	NGC 7662[4] NGC 3242[4]	3342	Nova Gem[6]	3342	Z And[15]
	auroral							
	$^3P_2 - {}^1D_2$	3875.4 ‖ §	3868.85 3967.62	Orion Neb.	3868 3969	Nova Per[2]	3868.73 3967.36	Z And[15]
	$^3P_1 - {}^1D_2$	3974.4 ‖ §	3868.83 3967.64	NGC 6572	3868 3969	Nova Gem[6]		
	nebular		3868.85 3967.57	NGC 6826	3868.4 3969.0	Nova Pic[3,16]		
Ne IV 95	$^2D^o - {}^2P^o$ auroral	4712 ± 10 *	4725.7	NGC 7027[4]	4722.5 4724	Nova Pic[3] Nova Per[17]		

Na IV	$^3P_2 - {}^1D_2$	3243	violet	violet	
99		† ‖			
	$^3P_1 - {}^1D_2$	3340	3346 NGC 7027[4]	?	3346 Z And[15]
		† ‖	3347 NGC 7662[4]		
	nebular				
Na V	$^2D^o - {}^2P^o$	4000	?	?	
135	auroral	†			
Si I	$^3P_1 - {}^1S_0$	6526.9	No	6525.3 Nova Pic[3,18]	
8.12	transauroral	*			
P II	$^1D_2 - {}^1S_0$	6690	?[19]	?[19]	
19.8	auroral	†			
	$^3P_1 - {}^1S_0$	4100	?[19]	?[19]	
	transauroral	†			
S I	$^3P_1 - {}^1S_0$	5365[20]	No	5376 Nova Gem[6,21]	
10.3	transauroral	*			
S II	$^4S^o_{1\frac{1}{2}} - {}^2D^o_{2\frac{1}{2}}$	6717	No	6726 Nova Gem[6]	
23.3		* §			
	$^4S^o_{1\frac{1}{2}} - {}^2D^o_{1\frac{1}{2}}$	6731	6731 NGC 6572[4]		
	nebular	* §	6729 NGC 7027[4]		
	$^4S^o_{1\frac{1}{2}} - {}^2P^o_{1\frac{1}{2}}$	4068	4068.7 } Orion Neb.[4]	4068.6 Nova Pic[3]	4068.56 η Car[23]
		* §	4076.6 }	Nova Aql[22]	4068.56 HD 4677
	$^4S^o_{1\frac{1}{2}} - {}^2P^o_{\frac{1}{2}}$	4076	4068.67 } IC 418[4]		4068.2 Z And[15]
		* §	4076.2 }		4068 See Note 25
	transauroral		4068.83 } NGC 6572[4]		
			4076.3 }		
S III	$^1D_2 - {}^1S_0$	5020	5017 NGC 6572[4,26]		
34.9		†	5018 NGC 7027[4]		
	auroral				
	$^3P_1 - {}^1D_2$	7887	red	red	
		† §			
	$^3P_2 - {}^1D_2$	8235	red	red	
	nebular	† §			

* From difference of permitted lines, $\lambda \pm 0.3$ except where otherwise noted.

† From Moseley extrapolation, may be uncertain by 200 Å.

‡ From series limits, some may be uncertain by 200 Å.

§ $\Delta\nu$ known, ± 3, from ultra-violet lines.

‖ Series limits of Ne II adjusted in the same manner as those of O II, as discussed in the text. This shift affects Moseley extrapolations for F II and Na IV.

[1] Wright, *Publ. Lick Obs.*, **14**, 38 (1925).

[2] Lockyer, "On Some of the Phenomena of New Stars," *Sol. Phys. Comm. Publ.*, 1914.

[3] H. S. Jones, *Cape Obs. Ann.*, **10**, No. 9 (1931).

[4] W. H. Wright, *Publ. Lick Obs.*, **13**, 1918.

[5] Paddock, *Astr. Soc. Pac. Publ.*, **31**, 47 (1919).

[6] W. H. Wright, *Publ. Lick Obs.*, **14** (1925).

[7] Not resolved in novae because of the width of Hα; probably present because of the great strength of Hα.

[8] J. C. McLennan, *Proc. Roy. Soc.*, **120A**, 327 (1928).

[9] P. W. Merrill, *Astr. Soc. Pac. Publ.*, **40**, 254 (1928).

[10] Measured on Harvard plates.

[11] Wright notes that the "nebular" line is probably present.

[12] The strongest unforbidden line of F II appears in Nova Pictoris at about the same time.

[13] Frequency difference correct, and accurately known, for the two Nova lines listed.

[14] Paddock, *Astr. Soc. Pac. Publ.*, **30**, 244 (1918).

[15] H. H. Plaskett, *Dom. Ap. Obs. Publ.*, **4**, No. 10 (1928).

[16] 3968 is blended with $H\epsilon$, but is undoubtedly very strong.

[17] Measured on Harvard plates.

[18] A somewhat doubtful ascription.

[19] Possibly masked by strong lines of He I and N III.

[20] Ruedy, Washington meeting, American Physical Society, April, 1933; abstract to be published in *Physical Review*, June 15, 1933.

[21] A possible identification.

[22] Miss Cannon, *Harvard Annals*, **81**, No. 3 (1920); the wave-length system is somewhat indefinite, but the lines are undoubtedly present.

[23] Moore and Sanford, *Lick Obs. Bull.*, **8**, 55 (1913).

[24] P. W. Merrill, *Contr. Mt. W. Obs.*, **355** (1928).

[25] P. W. Merrill, *Astr. Soc. Pac. Publ.*, **44**, 123 (1932); "Objects Intermediate between Planetary Nebulae and Stars."

[26] The identification is very doubtful. The nebular line is probably due to He I, 5015.7.

The identifications of forbidden lines in astrophysical sources, including those previously known, are summarized in table 2. Successive columns give the atom and its ionization potential (known or estimated), the type

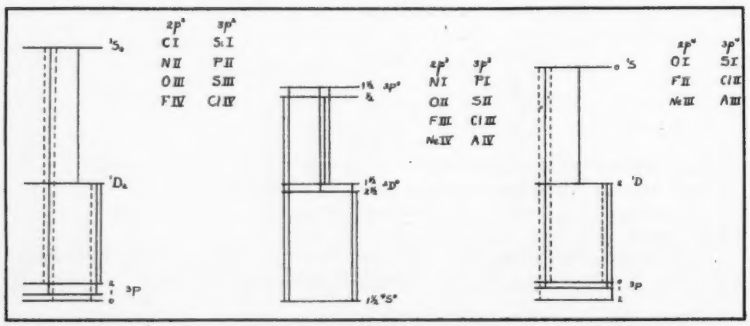

FIGURE 1

Schematic diagrams of low metastable configurations. Transitions are designated as follows in the text: middle to low, *a;* high to middle, *b;* high to low, *c.*

of transition, the predicted wave-length and a symbol indicating the method of calculation, three columns of observational data and references to the notes that follow the table. The references are representative

rather than complete. The astrophysical wave-lengths have not in all cases been reduced to the International scale, but the difference from the Rowland scale is much less than the probable error of the predictions.

The observational evidence on the relative intensities of nebular pairs is summarized in table 3. The table is divided into three sections, corresponding to the three configurations shown in figure 1. The intensities are in satisfactory accord with prediction.

TABLE 3

RELATIVE INTENSITIES OF NEBULAR PAIRS

ATOM	N II			O III	
Configuration	$2p^2$			$2p^2$	
	6548	6583		4958	5007
N.G.C.6543[1]	..	10	N.G.C.6543[1]	58	92 ±
N.G.C.6572[1]	10	30	N.G.C.6572[1]	75	120
+30°3639[1]	70	110	+30°3639[1]	8	10
			Z Andromedae[3]	2	5
			Nova Pictoris[2]	5	7
			Nova Geminorum[4]	4	5
ATOM	O II			S II	
Configuration	$2p^3$			$3p^3$	
	3727	3729		4068*	4076*
N.G.C.6572[1]	3	1+	N.G.C.6572[1]	6	3
+30°3639[1]	15	7	+30°3639[1]	60	20
I.C.418[1]	50	30	I.C.418[1]	20	5
			Z Andromedae[3]	2	..
			η Carinae[6]	1	..
			Nova Pictoris[2]	1	..
ATOM	O I			Ne III	
Configuration	$2p^4$			$2p^4$	
	6300	6363		3869	3968
N.G.C.6572[1]	3	1−	Orion Nebula[1]	40	30
N.G.C.7027[1]	5	2	N.G.C.6543[1]	40	30
H.D.50138[5]	medium	weak	N.G.C.6826[1]	40	25

* Transauroral pair.

[1] W. H. Wright, *Publ. Lick Obs.*, **13** (1918).
[2] H. S. Jones, *Cape Obs. Ann.*, **10**, Part 9 (1931).
[3] H. H. Plaskett, *Publ. Dom. Ap. Obs.*, **4**, No. 10 (1929).
[4] W. H. Wright, *Publ. Lick Obs.*, **14** (1925).
[5] P. W. Merrill, *Ap. J.*, **73**, 348 (1931).
[6] Moore and Sanford, *Lick Obs. Bull.*, **8**, 55 (1913).

Representative data on the relative behavior of auroral and nebular lines are given in table 4. The transauroral lines that have been observed are so few that we cannot generalize as to their behavior relative to nebular and auroral lines. From their occurrence in Nova Pictoris, and their coexistence with O III in Z Andromedae, the transauroral lines of S II seem to appear late and to be very persistent.

TABLE 4

RELATIVE INTENSITY OF AURORAL AND NEBULAR LINES

	N II		O I		O III		Ne III	
	5755	6547 6583	5577	6300 6363	4363	4958 5007	3342	3869 3968
	AUR	NEB	AUR	NEB	AUR	NEB	AUR	NEB
Nebulae*								
N.G.C. 6572	2	10	no	3	20	80	..	..
		30		1—		250	..	..
N.G.C. 7027	4	10	no	5	80	200	..	70
		30		2		800	..	70
N.G.C. 7662	..	..	..	..	15±	145	5	45
		..		..		360		40
+30° 3639	20	70	10	no	no	8	..	no
		110		..		10		no
N.G.C. 3242	..	..	..	..	15	123	71	5
Novae		..		..		184	..	40
Nova Vel ‖	..	..	..	..	100	10	..	..
		..		..		25		..
Nova Per ‖	..	..	..	..	80	2	..	..
		..		..		25		..
Nov Gem II ‖	..	..	..	..	50	2	..	..
		..		..		9		..
Nov Gem†	1	..	1	2	no	no	..	no
1918 March 18		..		1		no		no
Sept. 23	4	..	no	1—	5	5	1—	3
		..		1—		5		3+
Nova Pic‡								
1925 December	..	..	..	..	no	no	no	no
		..		..		no		no
1926 March	(5)	..	no	no	4	2	..	5
		..		no		4		1
Sept.	8	..	no	no	40	2	..	30
		..		no		10		30
1928	no	no	no	no	14	5	..	..
		no		no		7		..
Z And §	no	no	no	no	2	2	1—	2
		no		no		5		2
Aurora	no	no	strong	weak	no	no	no	no
		no		weak		no		no

* Wright, *Lick Obs. Publ.*, **13**, 242 (1918).

† Wright, *Ibid.*, **14** (1925); maximum took place about March 15.

‡ Jones, *Cape Obs. Ann.*, **10**, No. 9 (1931); maximum was before June 18; this nova developed with unusual slowness.

§ H. H. Plaskett, *Publ. Dom. Ap. Obs.*, **4**, No. 10 (1928).

‖ *Harv. Ann.*, **76** (1912).

So far as the first two rows of the periodic table are concerned, the identifications of tables 1 and 2 represent the most that can now be attained in the direction of discovering forbidden lines in astrophysical sources. The identifications of Ne III, Ne IV and F III seem the most certain, and are of interest in suggesting a fairly high abundance for these atoms.

[1] I. S. Bowen, *Astrophys. Journ.*, **67**, 1 (1928).
[2] I. S. Bowen, *Nature*, **123**, 450 (1929).
[3] I. S. Bowen, *Phys. Rev.*, **36**, 600 (1930).
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[10] I. S. Bowen, *Phys. Rev.*, **36**, 600 (1930).
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[12] C. H. Payne, these PROCEEDINGS, in press.
[13] R. S. Richardson, *Astr. Soc. Pac. Publ.*, **43**, 76 (1931), records the band spectrum of SiF in sunspots.
[14] J. C. Boyce and K. T. Compton, these PROCEEDINGS, **15**, 656 (1929).
[15] P. W. Merrill, *Mt. W. Contr.*, **460** (1933).
[16] H. N. Russell, K. T. Compton and J. C. Boyce, these PROCEEDINGS, **14**, 280 (1928).
[17] I. S. Bowen and D. H. Menzel, *Publ. A. S. P.*, **40**, 332 (1928).
[18] W. H. Wright, *Publ. Lick. Obs.*, **13** (1925), cf. discussion, p. 258, *et seq.*

LUMINOSITY DISTRIBUTION AND AVERAGE DENSITY OF MATTER IN TWENTY-FIVE GROUPS OF GALAXIES

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1. A survey of the inner parts of the metagalactic system was completed and published a year ago[1] in the form of a photometric catalogue of all external systems (excluding globular clusters) brighter than the thirteenth photographic magnitude. Extending the work to fainter systems, I have reported recently in a series of four papers on the results of the study of remote external galaxies.[2]

In the first of this series it is reported that the survey to the eighteenth magnitude, on the Harvard long exposure photographic plates accumulated during the past thirty years, has resulted in the finding of more than seventy-five thousand new galaxies, although only a little more than one-third of the southern sky and a twentieth of the northern have been thoroughly covered. The preliminary examination of the new data (second report) shows that the irregularities in distribution are large and, in high latitudes, independent of the distribution of faint stars; these irregularities are therefore probably not to be attributed to chance or to obscuring nebulosity, but rather are genuine, indicative of actual physical conditions and pointing to evolutionary processes in the metagalactic

system. In the third paper of the series it is shown how the faint high latitude cluster-type Cepheids are being used, in connection with the survey of external galaxies, to measure the extent of our own galactic system and to study especially its thickness perpendicular to the galactic plane. The fourth summarizes the study of the relation of the average number of galaxies in a square degree to the galactic latitude and longitude; it is found, on the basis of this material, that outside the wide galactic zone the concentration toward the galactic poles is inappreciable and also that the irregularities in distribution for galactic latitudes greater than $\pm$ 30° are so conspicuous as to be significant in considerations of the distribution of matter throughout the metagalaxy.

2. The average density of matter in metagalactic space is of importance in cosmogonic theory. In the study of the inner parts of the metagalactic system we deduced a value of 10^{-30} grams per cubic centimeter. This value is probably a minimum because no allowance is made for material outside the individual galaxies, and the estimated distances may be systematically somewhat too great. The largest uncertainty in the estimates lies, however, in the assumed mass of the average galaxy. It was taken as about 10^9 times the solar mass, but a value of one-tenth or ten times the assumed value is quite within reason. Until we have more certain estimates of the average mass of galaxies it will therefore be best to state the density in terms of the number of galaxies per unit volume; for example, the provisionally adopted value for metagalactic space can be expressed as 10^{-72} galaxies per cubic centimeter.

An important feature in the non-uniform distribution of galaxies is the occurrence of many physical groups. Within these groups the average density of matter must be much greater than the average for all space. In the present study I attempt to discover how great is the density in such regions and at the same time derive frequency curves of the total magnitudes in each group.

A dozen or more groups of galaxies are now known in which the members exceed two hundred in number. Some of these systems are spheroidal in form, centrally concentrated and of rather definite boundary. The majority of the groups, however, are of irregular form, with indefinite boundaries, and small in membership. Such groups are to the metagalactic system as the open star clusters are to the stellar system.

3. The observations and computations on the mean density of matter in twenty-five groups of galaxies are summarized in table 1. Galactic coördinates are given in the second and third columns, the groups being listed in order of increasing galactic latitude. The magnitude limit of the plate, for stars, is given in the fourth column; the limit for galaxies is on the average perhaps two-tenths of a magnitude brighter. By background density in the fifth column is meant the number of galaxies per square

degree, over an area of nine square degrees (minus the group), on the long exposure photographs used for the study of the various groups. The number varies from 5.7 for the first field, which is in such low galactic latitude that obscuration may affect it, to 77.8 per square degree for group 21.

TABLE 1

GROUPS OF GALAXIES

GROUP	β °	λ °	PLATE LIMIT m	DENSITY OF BACK-GROUND (PER SQ. DEG.)	RATIO OF DEN-SITIES (GR. TO BKG.)	AREA OF GROUP (SQ. DEG.)	GAL-AXIES IN GROUP	DIS-TANCE (MPC)	SIN θ	VOLUME (10^{-4} CU. MPC)	GALAXIES PER CC. (10^{-70})
1	+19	277	18.0	5.7	7.7	0.26	12	8.3	0.00349	1.0	37
2	+20	241	17.7	22.2	4.3	0.92	88	11	0.00873	37	8.1
3	−24.5	300	18.4	27.0	6.1	0.46	77	15	0.00582	28	9.4
4	+27	235	17.9	17.9	5.1	0.50	46	24	0.00582	114	1.4
5	+27	238	17.5	34.6	3.8	0.43	56	10	0.00436	3.5	55
6	−27	15	18.0	10.3	8.2	0.10	8	14	0.00145	0.35	68
7	−27	241	17.3	33.8	3.3	0.50	56	18	0.00436	20	7.1
8	+30	281	17.8	26.8	2.6	5.5	379	25	0.01454	2000	0.54
9	+31	295	17.7	20.8	11.3	0.20	46	16	0.00378	9.3	17
10	−34.5	309	18.8	21.4	2.8	0.97	58	22	0.00727	170	1.2
11	+35	282	17.6	20.4	6.8	0.33	45	22	0.00524	64	2.4
12	−38	308	18.8	21.4	2.8	0.64	39	28	0.00873	610	0.22
13	−42	229	17.5	61.8	2.1	1.14	150	26	0.01018	775	0.66
14	+43	357	17.4	46.5	3.7	2.17	370	23	0.01280	1030	1.2
15	+43	359	17.4	46.5	4.0	1.37	256	23	0.01076	635	1.4
16	+45	274	18.0	22.2	4.4	1.00	99	29	0.00756	440	0.69
17	−48	231	18.2	54.8	3.0	0.95	157	29	0.00873	680	0.78
18	−49	248	18.3	42.6	3.6	0.21	32	26	0.00291	18	6.00
19	−49	356	18.4	20.8	4.6	0.22	21	14	0.00204	1.0	63
20	−50	231	18.2	54.8	3.0	2.84	472	20	0.01309	2290	0.51
21	−51	234	18.0	77.8	1.9	0.46	66	29	0.00524	147	1.5
22	−53	209	17.7	55.6	3.1	0.33	58	28	0.00436	77	2.6
23	−72	86	18.2	23.4	4.2	0.84	82	33	0.00873	1000	0.28
24	−82	26	18.4	14.3	12.0	0.15	26	33	0.00262	27	3.3
25	−82	27	18.5	21.1	5.8	0.45	56	33	0.00465	150	1.2
Means			18.0	32.1	4.8		110	22.7			

The sixth column of table 1 gives the ratio of the number of galaxies in the group to the number in the background. The ratio falls below 3.0 for only five groups. With the area in the seventh column, and the background density, the probable number of actual group members has been determined and is given in the eighth column. The remainder of the table gives distance, volume and density for each group.

4. The distribution of luminosities is shown in table 2, and it is from a consideration of the brightest galaxies in each group or from the maxima

of the luminosity curves that the distances have been estimated. The luminosity diagrams for a few typical groups are shown in figure 1, based on this material. Table and figure both illustrate the diversity in magnitude distribution. In tabulating the frequency of magnitudes for each

TABLE 2

LUMINOSITY DISTRIBUTION IN GROUPS OF GALAXIES

GROUP	14.0	14.0 0.1	0.2 0.3	0.4 0.5	0.6 0.7	0.8 0.9	15.0 0.1	0.2 0.3	0.4 0.5	0.6 0.7	0.8 0.9	16.0 0.1	0.2 0.3	0.4 0.5	0.6 0.7	0.8 0.9	17.0 0.1	0.2 0.3	0.4 0.5	0.6 0.7	0.8 0.9	18.0 0.1	[18.1
1		1	1	2			1	1					1	3					1	1			
2		2	2	2	2		1	2		1	1	5	4	4	1	5	1	3	13	22	17		
3							1	1			1	3	3	1	1	8	7	4	12	10	7	18	
4											1	3	1	2	1	2	3	5	9	10	9		
5		2	2	1		1	1		1	3	2	2	5	5	8	3	2	5	7	6			
6								2	1	1			1	1			1				1		
7								1	2		5	5	2	6	3	5	16	4	7				
8					2		2	1	3	4	2	8	19	14	20	39	37	101	74	38	14	1	
9					1	1	1	3		5			2	3	7	7	2	5	4	4	1		
10											2	4		2	7	3	7	3	10	7	11		1	1
11								1			1	3	5	7	4	2	8	10	3	1			
12													1	1	2		1	2	5	2	6	9	5	5
13												2	10	3	3	21	26	22	46	17			
14											1	17	8	17	22	21	80	65	126	13			
15									3	1	10		9	9	17	16	34	47	88	22			
16											1		2	10	4	2	2	16	15	12	8	22	5	
17															6	7	12	18	31	25	30	23	5	
18														3		3	2	3	2	1	2	4	11	1
19									1	1	1	3	2	1	2	2	6	1	1					
20													1	1	12	40	31	48	82	58	88	82	29	
21							2			1	1		2	2	3	2	5	3	16	4	7	15	3	
22													2	5	1	4	8	3	8	15	12			
23														2	3	4	8	2	13	14	7	14	15	
24																	2	2		3	3	7	3	6
25							1	1						1	1	2	2	3	2	4	8	11	8	12

group the observed numbers have been corrected for background, the corrections distributed in accordance with the assumption of uniform density in space for background galaxies.

Among the twenty-five groups for which luminosity data are here presented two double groups and one triple system occur: Numbers 14 and 15, 24 and 25, and 17, 20 and 21. In table 1 it is seen that the members of the suggested triple group are found on plates showing an unusually high background density; it is possible that they are but nuclei or concentrations in a very extensive canopy of galaxies.

5. A discussion of the data given by Hubble and Humason[3] on the photographic magnitudes of individual stars in the nearer galaxies, together with the Harvard photographic magnitude survey for the same objects, has led to the value -14.5 for the mean absolute photographic magnitude of a galaxy. An independent treatment of the published data

has led Knox-Shaw, in his presidential address to the Royal Astronomical Society, to deduce the same value.[4] Following Hubble and Humason's method, I compute from this revised value a coefficient of 405 km./sec. per megaparsec as the rate of the recession of galaxies.

For only a few of the groups studied on the Harvard plates is it possible to estimate accurately either the median apparent magnitude of the galaxies in a group or the maximum in the frequency curve of magnitudes. The groups extend below the plate limits. For a majority there is, however, a preliminary maximum in the luminosity curve. The absolute magnitude of these brightest nebulae has been taken as -16.0, probably a conserva-

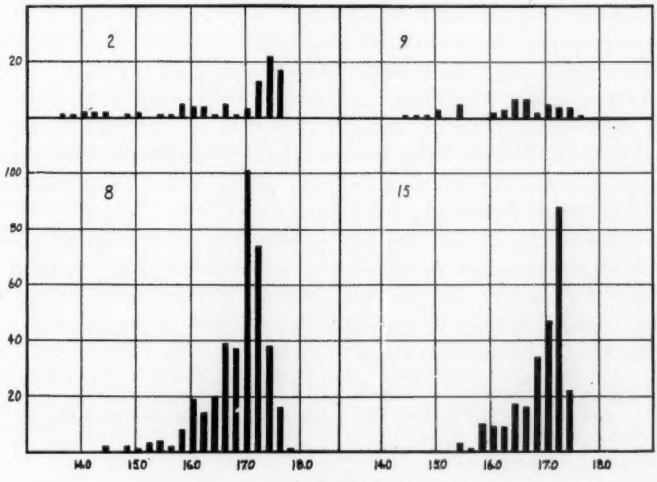

FIGURE 1

Luminosity distribution in four groups of galaxies. Abscissae are photographic magnitudes; ordinates are numbers of galaxies.

tive estimate. It is based not only on the present material but also on the luminosity curves for the richer groups as studied at Harvard and Mount Wilson.

6. For several groups the volumes for which the densities in the last column of table 1 were computed are somewhat less than would have been obtained from the areas given in the seventh column. The adjustment permits the determination of the mean density for only the principal parts of those groups having very irregular form and indefinite boundaries. The sine of the mean angular radius, θ, used in computing the density, is given in the tenth column, the volume being $\frac{4}{3}\pi R^3 \sin^3\theta$, where R is the

distance in megaparsecs. The number of galaxies entering the computation of densities is, of course, appropriately corrected to the revised dimensions.

The volumes and densities are seen to differ greatly from group to group. The smallest densities appear to be but twenty or thirty times that of metagalactic space; but it must be remembered that they are minimum values, especially in the more distant systems where only the brightest members are shown on the Harvard plates. On the grounds of population alone, the tabulated density should be increased for the remoter systems by a factor that may be anywhere between two and ten. Errors in the estimates of distance will enter as the cube in the density, indicating the considerable uncertainty in the values for individual groups.

A computation of the mean densities in the eight groups of galaxies listed by Hubble and Humason in Mount Wilson Contribution 427 gives results comparable with those in table 1; all the values fall within the range here found, with an average density for the six richest groups of about 9×10^{-70}. For these groups the total estimated population has been used in the computation and not, as in table 1, only the partial membership that is actually observed.

In conclusion, it seems that we can take 5×10^{-70} galaxies per cubic centimeter as the approximate mean density of matter in space for a large number of groups of galaxies, and note that in a few relatively small groups, where the individual galaxies are nearly in contact, the mean density may be ten times as great.

[1] Shapley and Ames, *Harv. Annals*, **88**, No. 2 (1932).

[2] *Harv. Obs. Bulletin* **889** (1932); *Ibid.*, **890** (1932); these PROCEEDINGS, **19**, 29–34 (1933); *Ibid.*, **19**, 389–393 (1933).

[3] *Mt. Wilson Contr.* 427 (1933).

[4] *Mon. Not. R. Ast. Soc.*, **93**, 304 (1933).

ABSORPTION AND SPACE REDDENING IN THE GALAXY FROM THE COLORS OF B-STARS

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The stars of spectrum class B, with their great intrinsic luminosities, furnish a means of testing the transparency of space. The present work grew out of a conversation in the autumn of 1930 with Dr. R. J. Trumpler, who afterward kindly furnished us with a list of B-stars which promised to reveal some of the space reddening which he had found in a study of open clusters.[1] The observing list finally grew to include all stars north of declination $-15°$, brighter than visual magnitude 7.5, and of classes B0 to B5 in the Draper Catalogue, about 700 objects. When a revised classification by Plaskett and Pearce[2] and others became available it was found that a considerable number of O-stars as well as some of spectra as late as B8 to B9 had been included.

The method was the same as has already been described for the globular clusters.[3] With a photoelectric cell attached to the 15-inch refractor at Madison, each object was measured through two filters, which with the cell used have effective sensitivities at wave-lengths 4200 Å and 4700 Å, giving a difference in color-index between A0 and K0 of 0.74 mag. This value is just one-half the 1.48 mag. of Seares's visual-photographic scale for giant stars. Our calibration is based upon more than one hundred stars of spectra A to K in galactic latitude greater than 10°. The normal colors of B-stars were taken as the means of stars brighter than visual magnitude 6.0, and more than 15° from the galactic equator. Some of these standard objects may be appreciably affected by space reddening, but the effect is not serious. The adopted values for normal color are as follows:

SPECTRUM	COLOR-INDEX M
O	−0.23
B0	−0.22
B5	−0.17
A0	−0.10
A5	+0.03
F0	+0.09
F5	+0.20
G0	+0.33
G5	+0.49
K0	+0.64
K5	+0.90

Considerable care was spent on the determination of the atmospheric

extinction, but as the results depend upon differences of color between stars not far apart in the sky, the effect of the atmosphere is largely eliminated.

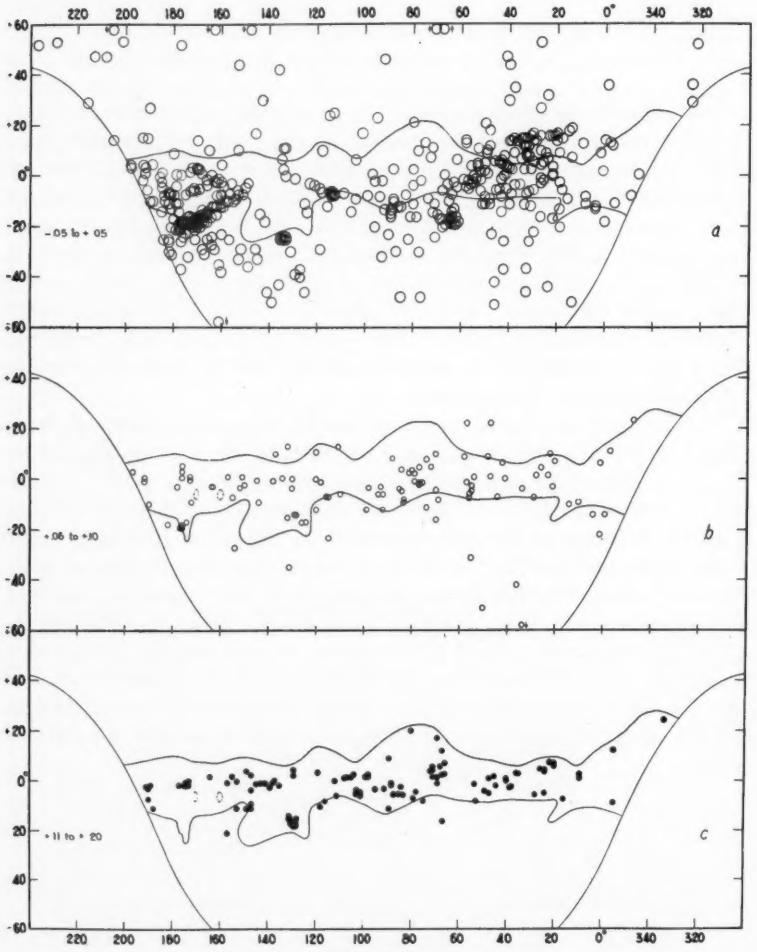

FIGURE 1

Color-excesses of B-stars.

We are indebted to Dr. Edwin P. Hubble for his unpublished determination of the zone of avoidance of extra-galactic nebulae, based upon a survey of many thousand objects at Mount Wilson. The irregular con-

tours marked in figures 1–3 show the region near the galactic equator within which no such nebulae are found, presumably because of the obscuring effect of dark material in our own galaxy. The boundaries are somewhat indefinite; the nebulae are most numerous near the galactic poles, and at any longitude they decrease toward the equator until a limit is reached beyond which no such objects are photographed. Within the zone of avoidance there are several small regions where Hubble finds a

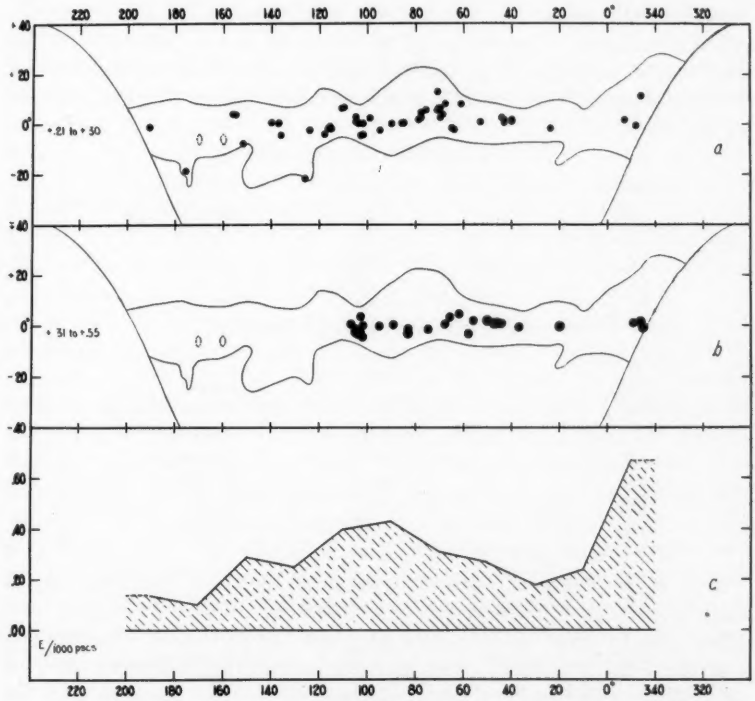

FIGURE 2

a and *b*. Color-excesses of B-stars. *c*. Space reddening at different longitudes.

few nebulae, and two such "holes" are marked at latitude $-6°$, longitudes $160°$ and $170°$. The pole of the galaxy is adopted at right ascension $12^h\ 44^m.4$, declination $+26°.8$ (1900).

We have distributed the colors of the B-stars according to shells of increasing distance from the sun, using mean absolute magnitudes, but this procedure is hampered by the rather large dispersion of the absolute magnitudes within each sub-class of spectrum. It is more satisfactory to

chart the objects according to color and independently of magnitude or distance, as has been done in figures 1–3.

These figures will be discussed in detail in a more extended publication, but a number of main features are obvious. We call the observed color-excesses from −0.05 to +0.05 mag. as normal, +0.06 to +0.10 as suspicious and greater than +0.10 as definitely colored. On this basis the normal stars in figure 1*a* are well distributed and extend to high latitudes, while the colored objects, figures 1*c*, 2*a*, 2*b*, are practically all within the zone of avoidance of the nebulae, the reddest stars being close to the galactic equator. Comparing the different diagrams, there are regions which stand out with all the stars normal, others with all of them colored. The approximate positions of a number of such regions may be indicated as follows:

REGIONS WITH NO REDDENING

LONGITUDE	LATITUDE	CONSTELLATION
30°	+15°	Cygnus-Lyra
64	−17	Lacerta
134	−23	Pleiades
170	−17	Orion

REGIONS WITH STRONG REDDENING

LONGITUDE	LATITUDE	CONSTELLATION
346°	0°	Aquila
46	+ 2	P Cygni Region
70	+ 5	Cepheus
84	− 3	Cassiopeia
104	− 2	Near Perseus Cluster
129	−17	Perseus-Taurus
140	− 1	Auriga

It is by no means the case that the areas of strong reddening appear dark in the sky. For instance, the region near P Cygni includes a well-known bright cloud of stars, most of which are undoubtedly between us and the colored B-stars. The material causing the reddening may be beyond the star cloud, or, what is more probable, the material is in the spaces between the stars. In general, since the apparent reddening follows closely the line of the Milky Way, it is reasonable to look for the largest effects of coloration not where there are apparently few stars but where there are many.

In figure 2*b* it is seen that there are no stars with color-excesses greater than +0.30 in the whole region from longitude 110° to 200°. As this area includes the anti-center of the galaxy at longitude about 150°, and includes also the "holes" detected by Hubble, it would seem that the B-stars of magnitude 7.5 extend to the outer spaces of the galaxy in that direction, at least so far as scattering material is concerned. This con-

clusion is strengthened by the colors of a score of B-stars in longitude 115° to 200° averaging about magnitude 8.5, measured with the reflectors at Mount Wilson. Not one of these fainter objects shows a color-excess greater than 0.30 mag.

For a further test of the variation of the space reddening with longitude, the stars within 5° of the galactic equator were grouped in sections of 20° of longitude, and on the basis of mean absolute magnitudes the average color-excess per thousand parsecs of distance was determined. Due to the apparently spotted nature of the effect, the results depend largely upon how the averages are taken. As a first trial the computed distances were simply added, and this total length of path was divided into the sum of the color-excesses for the corresponding stars. The results are shown in figure 2*c*. While no great weight is given to the numerical values, the relative distribution of the space reddening is undoubtedly real, there being a maximum near 90°, low values near the anti-center, and strong reddening at 340° as we approach the galactic center. For the values of 0.10 mag. per thousand parsecs at longitude 170° and a maximum color-excess of 0.30 mag., there follows the distance of 3000 parsecs as the limit of dark material in that region.

The galactic center is too far south to be reached satisfactorily from Madison, but we may compare the present results with the measures of globular clusters taken at Mount Wilson. In figure 3 the color-excesses of the globular clusters have been reduced to the same scale as that of the B-stars, and it is seen that the reddened clusters are all in the general region of obscuration. The zone 7° wide where no clusters are found is indicated by the shading, and in this narrow strip are the five B-stars which show the greatest reddening in that neighborhood, their color-excesses ranging from 0.35 to 0.52 mag. There are a dozen or more clusters not yet observed, but from the figure as it stands it seems reasonable to infer that Hubble's limits definitely outline the nucleus of the galaxy in longitude not far from 330°, and that the globular clusters concentrated in the region are subject to reddening and absorption which increase toward the galactic plane until finally no clusters are visible. The strongly reddened B-stars are presumably in the foreground of the picture. Undoubtedly a test of faint B-stars in low latitude near the center will show even greater reddening, and if a few globular clusters can be found in the apparently forbidden region, the maximum of coloration may be anticipated. It is expected to carry out these tests during the next summer at Mount Wilson.

In Shapley's general system of globular clusters the positions in space of a number of the members are placed nearly in line with but far beyond the nucleus of the galaxy. If this view is correct, how is it that the light of the clusters comes through where the extra-galactic nebulae are blotted

out altogether? The total light of the faintest cluster is equivalent to about the thirteenth photographic magnitude, and nebulae several magnitudes fainter could easily be detected if they were in the same apparent direction. We are therefore convinced that the general region near the galactic center is opaque to the light of objects beyond, and that the faint globular clusters in that direction are between us and the center. It is

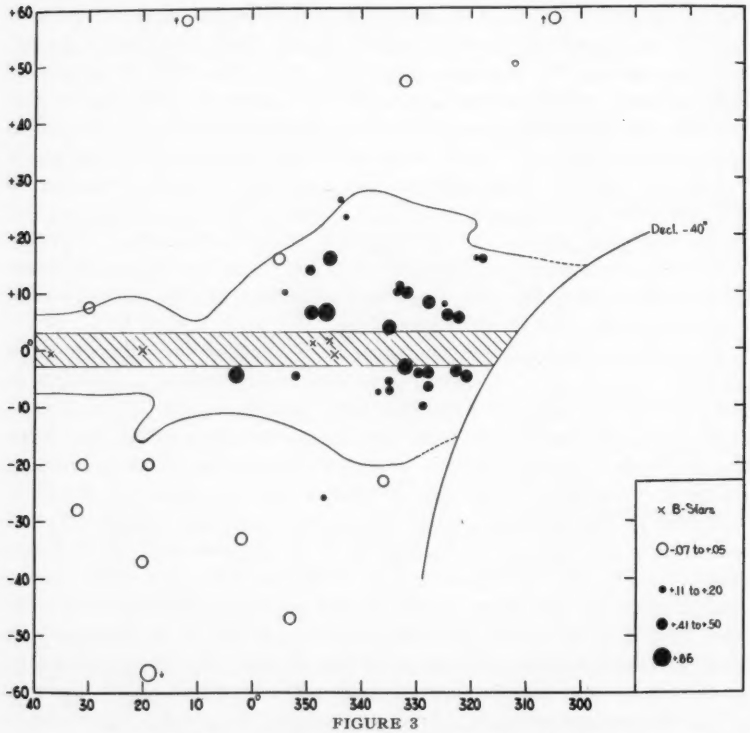

FIGURE 3

Color-excesses of globular clusters near the center of the galaxy.

obvious that the assumption that the clusters are all this side of the nucleus increases again the inferred size of the galaxy, perhaps doubling the diameter.

Because of the dispersion in their intrinsic mean colors it is not feasible to derive individual distances from the color-excesses of the globular clusters, as is perhaps possible for the B-stars. Moreover, it is by no means clear that all of the space absorption is indicated by the selective scattering, which would be due only to particles small in comparison with

the wave-length of light. Van de Kamp has made a series of attempts to correct the distances of the globular clusters on the basis of a uniform absorbing layer near the plane of the galaxy. In his last paper[4] the optical thickness of 0.8 photographic magnitude is adopted, which gives a reducing factor of 30 or more for the distances of clusters in low latitude. When his positions in space are charted to scale for the clusters near the nucleus in our figure 3, it is evident that while van de Kamp is on the right track his corrections are still far from final. Instead of being condensed into something like a spherical region of space, the clusters are drawn out in a line between the sun and the nucleus, showing that the distances are still doubtful. The difficulty probably comes from the assumption of a uniform absorption at all longitudes, whereas toward the nucleus it would be natural to expect an increase in density of both dark and luminous material. As previously noted, the increase of the dark material is shown in figure 2*c*.

As a test of another character we have compared our colors with the intensities of the interstellar calcium lines for several hundred stars listed by Plaskett and Pearce,[5] but there seems to be no correlation whatever between color-excess and calcium intensity. This result confirms that of Elvey and Mehlin[6] from about fifty stars in a region in Cepheus. As they point out, the space reddening cannot be due to material which is coextensive with the calcium atoms. The apparently complete independence of the calcium absorption and the space reddening is a matter for further investigation.

It is well known that the assumption that the space reddening of stars is caused by selective scattering of light from small particles leads to a great calculated mass of the material concerned. The problem has been studied by Pannekoek,[7] Eddington[8] and others. They were troubled by the high mass given by a limited absorbing region in Taurus, less than 2000 cubic parsecs in volume, but now we have the whole mid-galactic layer filled with stuff and in a volume probably millions of times greater than the space in Taurus. In the present state of the observational evidence it is premature to give any exact figures, especially as the amount of absorption in addition to that accompanied by reddening is still very doubtful. For illustration, however, we consider the total scattering of the dark layer in the direction perpendicular to its plane to be of the same order as the scattering of the earth's atmosphere. The air molecules which we look through at the zenith add up to 1000 grams per square centimeter. With this mass per unit of cross-section and a circular diameter of 30,000 parsecs (1 parsec = 3×10^{18} cm.), the total for the layer comes out 6×10^{48} grams or 3×10^{15} times the mass of the sun. As shown by Schoenberg,[9] the number of solid particles required to produce the same scattering would be much smaller, perhaps by a factor of $3 \times$

10^6 giving a mass of 10^9 suns, to be multiplied, however, by the ratio of the mass of the average solid particle to that of an air molecule. On dynamical considerations Plaskett[10] estimates the total mass of the galaxy to be 2.5×10^{11} suns which is considerably greater than the maximum number of stars, 3×10^{10}, estimated by Seares and van Rhijn. All of these estimates are quite uncertain, but on any calculation it will not be surprising to find the dark material greater in mass than the total of the stars.

The results then of the present study are that the reddening of the B-stars confirms the conclusion that our galactic system is filled near its median plane with a layer of dark material, which reddens all stars at sufficient distances and blots out everything behind it. The presence of this dark region is alone a good reason for concluding that our own galaxy is simply one of many similar systems which we can observe. Calculated distances of objects in low galactic latitudes must be revised with allowance for absorption, and the globular clusters in the direction of the nucleus are probably all on this side of the galactic center. Finally the dark matter in our galaxy may well be greater in total mass than the luminous material which we see in the form of shining stars.

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PRELIMINARY STUDIES OF CHROMOSOME RINGS IN BRODIAEA LACTEA[1]

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Brodiaea lactea is a liliaceous species which is propagated chiefly by offsets from an underground corm. The genus is almost unknown cytologically. Müller[3] reported the diploid chromosome number of an unknown species of *Triteleia* (*Brodiaea*) as 10–12. Johansen[4] recently reported the diploid numbers of four species of *Hookera* (*Brodiaea*) including *H. californica* (10), *H. stellaris* (12), *H. minor* (14) and *H. coronaria* (42).

A relatively small amount of material has been available for the present study, and the chromosome number is not yet finally determined. Counts indicate that 21–24 pairs of chromosomes are present. Some of the material used was collected from plants growing in the vicinity of Pullman, Washington;[5] other plants were grown from corms obtained from Theodore Payne, Los Angeles, California.

Apart from the usual pairs, three types of chromosome associations have been observed in the heterotypic prophases and equatorial plate: a ring or chain of four small chromosomes (Fig. 1*c–e*), a ring or chain of four large chromosomes (Fig. 2) and a ring or chain of six (Fig. 3). Cross-shaped configurations appearing in the prophases (Fig. 1*a* and *b*) suggest that the ring of four small chromosomes is the result of a reciprocal translocation.

Two hundred microspore mother cells were examined to note the frequency-distribution of the three types of chromosome associations. Since the counts were made from sectioned material, they cannot be considered as accurate as though a smear method had been used.

4 SMALL CHROMOSOMES	4 LARGE CHROMOSOMES	2 GROUPS: 4 LARGE AND 4 SMALL	6 CHROMOSOMES	2 GROUPS 6 AND 4 SMALL
17	63	13	105	2

The association of four small chromosomes is difficult to identify because of the similarity of these chromosomes in size to many of the other chromosomes in the cell. In most cases adjacent chromosomes in these complexes of four or six move to opposite poles as is the case in *Oenothera* and in certain races of *Datura*.[6] Chiasmata are usually terminalized before or during diakinesis so that any non-disjunction that occurs is not influenced by sub-terminal or interstitial chiasmata such as Sax and Anderson[7] found in *Tradescantia*. An analysis of the methods of separation of the chromosomes in 215 associations is given in table 1.

TABLE 1

	LARGE RING OF 4	LARGE CHAIN OF 4	SMALL RING OF 4	SMALL CHAIN OF 4	RING OF 6	CHAIN OF 6
Adjacent to opposite poles	35	18	21	8	81	11
Adjacent to same pole	5	0	2	1	11*	1*

* Each of these associations shows some adjacent chromosomes directed toward the same pole and some toward opposite poles of the spindle as is shown in figures 3*c* and 3*e*.

In all rings or chains of four in which adjacent chromosomes move to the same pole, chromosomes of unlike size always move together (Fig. 2*d*). In three cases not listed in table 1, groups of six chromosomes are so oriented that four chromosomes would apparently move to one pole

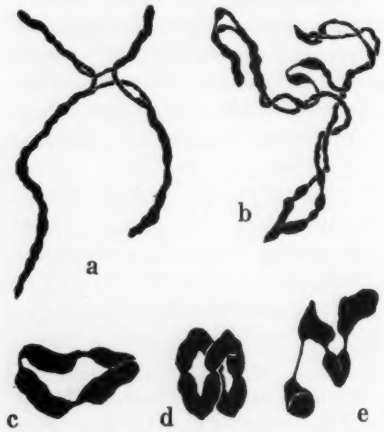

FIGURE 1

FIGURE 2

Figure 1.—Associations of four small chromosomes at various stages of the heterotypic division in microspore mother cells: *a*, cross-shaped figure at pachytene stage which shows an exchange of partners; *b*, same at early diplotene stage; *c*, ring at diakinesis; *d* and *e*, ring and chain at equatorial plate with adjacent chromosomes directed toward opposite poles of the spindle. × 2800.

Figure 2.—Associations of four large chromosomes: *a*, ring at diakinesis; *b* and *c*, ring and chain with adjacent chromosomes directed toward opposite poles; *d*, ring with adjacent chromosomes of unequal size directed toward same pole. × 2800.

and two to the other. A ring of this type is shown in figure 3*b* and a chain of similar nature in figure 3*g*. However, in some cases at least, this configuration may not result in the expected distribution of chromosomes. The arrangement shown in figure 3*e* seems to have been derived from a configuration similar to that shown in figure 3*b*. The orientation shown in figure 3*c* would result in complete disjunction and hence fertile

combinations in the microspores if the chromosomes are arranged as numbered (see Fig. 4*B*).

There are three facts which make it seem probable that the association of four large chromosomes is derived from the group of six by the loss of one pair from the complex. (1) In the two cases in which groups of six and four were found in the same pollen mother cell, the four was obviously the association of four small chromosomes (Fig. 3*f*). (2) In 105 cells, each with a group of six chromosomes, no indication was found of an association of four large chromosomes. (3) The chromosomes in the

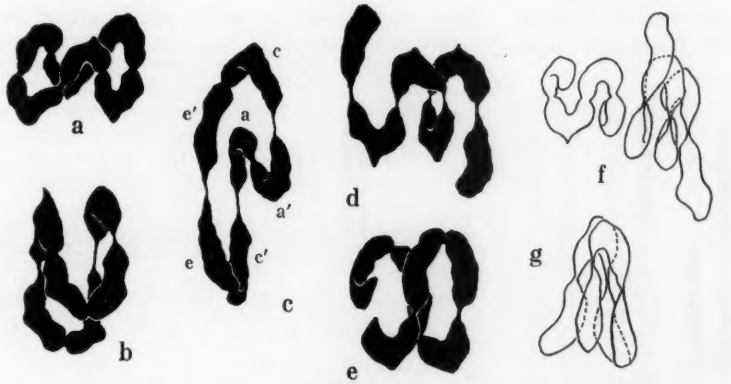

FIGURE 3

Associations of six chromosomes on heterotypic equatorial plates: *a*, ring with adjacent chromosomes directed toward opposite poles; *b*, ring with four chromosomes directed toward one pole and two toward the other; *c*, ring with chromosomes so oriented as to result in complete disjunction (see Fig. 4*B*); *d*, chain with adjacent chromosomes directed toward opposite poles; *e*, complete disjunction as in *c*, possibly derived from a configuration similar to that shown in *b*; *f*, chain of six and chain of four small chromosomes on same equatorial plate; *g*, chain with four chromosomes directed toward one pole and two toward the other. × 2800.

group of four large chromosomes are of approximately the same sizes as are those of two pairs in an association of six. If the ring or chain of four large chromosomes is derived from the group of six, the facts just noted indicate that in about 76% of the microspore mother cells three pairs of chromosomes are combined in a group of six; whereas in 24% of the cases the same chromosomes form a group of four and a "pair."

Since configurations of six chromosomes have not yet been observed at the pachytene stage, any suggestion as to why this group breaks apart in about a fourth of the pollen mother cells must be hypothetical. A tentative hypothesis to account for this behavior is indicated diagrammatically in figure 4. If the interchange between chromosomes *AB* and

CD involved approximately half of each chromosome, and that between the resultant *AD* chromosome and the *EF* chromosome involved only a small portion of each (Fig. 4*A*), the configuration shown in figure 4*B* would appear at the pachytene stage. With the large number of strands present in these microspore mother cells, mechanical difficulties might in some cases prevent the smaller interchanged segments from pairing with their homologues. This would result in an association of four chromosomes and a "pair." The only deficiencies in these associations would be the smaller translocated segments. A few groups of four large chromosomes have been observed with what might be interpreted as a sub-terminal chiasma. However, since nearly half of the length of the *CD* chromosome is paired with its homologue in the region *Y–Z*, it seems

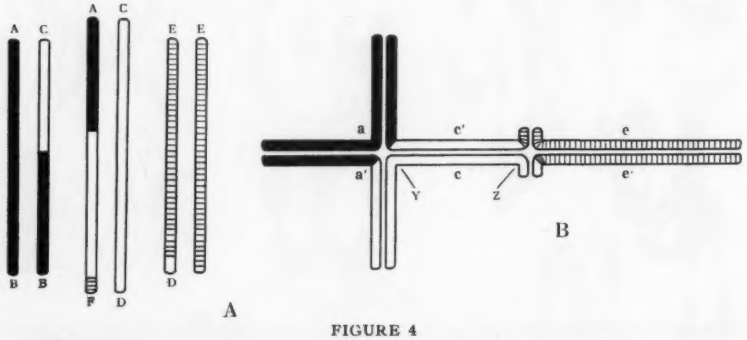

FIGURE 4

Diagrammatic representation of suggested explanation of the behavior of the chromosomes in the association of six: *A*, possible relation of interchanged segments; *B*, configuration which would be expected to occur at the pachytene stage as a result of the reciprocal translocations postulated in *A*.

possible that the terminalization of chiasmata formed in this region might result in the attachment of *D* and *F* though they are not homologous ends. More data are necessary to test adequately this hypothesis.

Since in practically all cases adjacent chromosomes move to opposite poles, or the groups of six separate as shown in figure 3*c*, all or nearly all spores except those formed from pollen mother cells with an association of four large chromosomes would be viable. Only half of this latter class would have the full complement of genes, on the assumption that there is no relation between the segregation of the group of four and that of the separate "pair." The direction of movement of the *ED* chromosome would determine whether the spores resulting from these divisions have the full haploid constitution. If this chromosome moves at random with respect to the *AF* chromosome, it is to be expected that the full

chromosome complement will be found in half of these spores. Thus about one-eighth of the total spores would be deficient for this small segment. Pollen has been examined from only one plant and all of it appeared to be morphologically perfect. Its germinability, however, is not known. It may be that the small deficiency indicated in the hypothesis here outlined would not be lethal to the microgametophyte. Since the plants are propagated vegetatively, these chromosome complexes may be carried on indefinitely.

[1] The author wishes to express his appreciation to Dr. C. E. Allen for valuable suggestions and criticisms.

[2] Research Associate in Botany under a grant from the Wisconsin Alumni Research Foundation.

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ABSENCE OF THE CORPUS CALLOSUM AS A MENDELIZING CHARACTER IN THE HOUSE MOUSE

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In a previous paper[1] there was reported the discovery of a cerebral variation of the house mouse characterized by complete absence of the corpus callosum which is the neocortical commissure normally present in all mammals above the Monotremes. Although a survey of numerous domestic mouse stocks has been made, the variation has been found only among descendants of a cross between rodless and silver strains, designed to determine the strength of linkage existing between the genes determining these two characters. Two cages yielded only normals, nine contained both normals and abnormals while samples from the remaining four cages consisted solely of animals lacking the corpus callosum. Enough data were available to show that the character was not sex-linked and that it was not another manifestation of the rodless gene itself.

Because no behavioristic peculiarity has been found correlated with absence of the corpus callosum, diagnoses of the character have been made by examination of individual brain sections. The necessity of sacrificing each individual diagnosed has rendered the genetic investigation laborious

but in spite of this handicap, sufficient data have been assembled from controlled matings to make certain the mode of its inheritance.

Individuals (usually males) were selected from cages shown to have contained animals lacking the corpus callosum. These were mated to individuals of normal unrelated strains. After the hybrid litters had been raised to weaning age the parents were killed and diagnosed, as were samples of their offspring. From matings of abnormal mice with normals there resulted 54 normal F_1 offspring, which proves the exceptional brain organization to be recessive. From matings in which the parent from abnormal stock died and was not diagnosed, there resulted 21 normals. In this latter mating the undiagnosed parent might have carried two, one or no genes for absent corpus callosum. Yet it is certain, as we shall see that some of these 21 normal progeny carried as a recessive the gene for absent corpus callosum.

Certain heterozygous individuals resulting from these outcrosses were mated *inter se* and produced an F_2 population of 36 animals, 28 of which were normals and 8 of which lacked the corpus callosum. Outcrosses in which the parent from abnormal-producing stock died without diagnosis, yielded in the F_2 13 normals and 4 abnormals. A few matings in which the P_1 parent was normal, but heterozygous for the abnormal gene, produced abnormals in the F_2. This population consisted of 18 normals and 4 abnormals, giving an F_2 total of 59 normals and 16 abnormals where the expectation for a recessive unit-character is 56.3 normals and 18.7 abnormals.

Several matings were made between mice from families known to have produced individuals lacking the corpus callosum. The results of these matings may be readily classified and are found to be fully in accord with the hypothesis that the aberrant character represents the developmental manifestation of a single recessive gene.

Abnormals bred *inter se* are recorded as having produced 1 normal and 10 abnormals. The exceptional normal should not have appeared, if the abnormality is a recessive character and we prefer to explain its occurrence as due to the accidental transfer of an individual from another cage or as a case of faulty diagnosis, rather than as a genetic irregularity. Five other matings in which both parents lacked diagnosis, produced a total of 29 abnormals and no normals.

Where both classes of young appeared as a result of mating abnormals with normals, there were recorded 20 normals and 21 abnormals. Equality is expected upon the basis of a single recessive gene in such back-cross matings. In matings in which only one class appeared there were produced 7 normals.

Where both classes resulted from the mating of abnormals with undiagnosed individuals (doubtless normal), there were recorded 13 normals

and 21 abnormals. Where only one class was produced there were found 7 abnormals, the undiagnosed parent presumably in this case having been abnormal.

When normals were mated to normals there resulted 12 normals. When normals were mated to undetermined individuals there were produced 12 normals and 20 abnormals. The results of these matings are summarized in table 1, in which it will be seen that F_1 outcrosses produced

TABLE 1

TYPE OF MATING	PARENTS	OFFSPRING NORMAL	OFFSPRING ABNORMAL
F_1 Outcross	Abnormal × normal	82	0
F_2 Outcross	Normal (heterozygous) × normal (heterozygous)	59	16
Backcross	Abnormal × normal (heterozygous)	45	62
Inter se	Abnormal × abnormal	1*	46

82 normals, F_2 outcrosses produced 59 normals : 16 abnormals, backcrosses produced 45 normals : 62 abnormals and *inter se* matings produced 46 abnormals and the one exceptional normal (starred) previously explained in the text.

The results of none of these matings are contrary to the interpretation that absent corpus collosum is due to the expression of a single recessive mendelizing gene, which so far as we have been able to observe, confers no detriment upon the individual possessing it. Studies are being made to test the linkage relationships of the gene for absent corpus callosum with the other unit-characters of the house mouse.

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THE TOPOLOGY OF INVOLUTIONS

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An extensive theory of the topology associated with finite groups of continuous transformations remains to be developed. Mr. M. Richardson has evaluated the Betti numbers of the "domain of discontinuity" in a case of considerable generality and his methods[1] yield equally well certain modulo p invariants. They seem to be inadequate, however, when p equals σ, the order of the group. We shall briefly develop the theory in the simplest non-trivial exceptional case, namely, $p = \sigma = 2$. Here we have a topological involution T operating in S. If each point of S is identified with its transform, the topological invariants of the resulting S_T are in a sense characteristic of T; for if T is deformed to the involution T' through a continuous family of involutions, then S_T' is homeomorphic with S_T. Our task is to study the mod 2 invariants of S_T. As an application we shall evaluate the invariants of symmetric product spaces.

1. We shall take for S a connected n-complex and shall deal only with mod 2 topology.[2] We assume that S admits a simplicial subdivision into K_n such that

(a) T merely permutes the cells of K.

(b) If a cell of K is invariant, it is pointwise invariant.[3]

The invariant cells form a subcomplex K^0 and the non-invariant p-cells of K can be grouped in pairs such that the two cells of a pair are transforms of each other. Thus if $TE_p^i = \bar{E}_p^i$, the p-cells of K are E_p^{0i} (in K^0) and E_p^i, $\bar{E}_p^i$. If $C = x_i E_p^i$,[4] we define $\bar{C} = TC$ to be the chain $x_i \bar{E}_p^i$.

Consider now a minimal base (Γ^i) for p-cycles, and let the transformation homologies be

$$\bar{\Gamma}^i \sim g_{ij}\Gamma^j (i, j = 1, \ldots, r;\ r = R_p(K, 2)).$$

Let $u = g + e$ where $g = (g_{ij})$ and e is the identity matrix of the same order. Also let $G^i = \Gamma^i + \bar{\Gamma}^i$. Since

$$G^i = \Gamma^i + \bar{\Gamma}^i \sim \delta_{ij}\Gamma^j + g_{ij}\Gamma^j = u_{ij}\Gamma^j$$

we may suppose that $G^1, \ldots, G^\rho$ are independent, where ρ is the rank of u.

Let (D) be the totality of p-cycles D such that $D + \bar{D} \sim 0$. If $D \sim x_i\Gamma^i$, then

$$0 \sim D + \bar{D} \sim x_i u_{ij}\Gamma^j$$

and hence $x_i u_{ij} = 0$. Conversely every solution of this system yields a D. Hence a minimal base for (D) is of order $r - \rho$. The cycles $G^1, \ldots,$

G^ρ are in (D) and we can now choose $r - \rho - \rho$ additional D's say D^1, $\ldots$, $D^{r-2\rho}$, which with the G's form an independent set. The cycles

$$(\Gamma) \qquad \Gamma^i, \bar{\Gamma}^i, D^j \ (i = 1, \ldots, \rho;\ j = 1, \ldots, r - 2\rho)$$

are independent; for if

$$L = x_i\Gamma^i + \bar{x}_i\bar{\Gamma}^i + y_jD^j \sim 0,$$

then

$$L + \bar{L} \sim (x_i + \bar{x}_i)(\Gamma_i + \bar{\Gamma}_i) \sim 0$$

and $x_i = \bar{x}_i$ with the result that

$$L = x_i(\Gamma^i + \bar{\Gamma}^i) + y_jD^j = x_iG^i + y_jD^j \sim 0$$

which is impossible. Thus the cycles (Γ) are a minimal base and the matrix of transformation homologies is now of a canonical form

$$\begin{matrix} 0 & I & 0 \\ I & 0 & 0 \\ 0 & 0 & I' \end{matrix}$$

where I and I' are of orders ρ and $r - 2\rho$. We shall suppose such a base to have been chosen for each dimension.

2. A chain X will be called invariant if $X + \bar{X} = 0$. If in particular every cell in X is in K^0, we attach an index 0, e.g., X^0, or X^{0i}_ρ. Every invariant chain can be written in the form $Y + \bar{Y} + Y^0$. By continuity, $\overline{F(X)} = F(\bar{X})$.[5]

2.1. If $H + \bar{H} + H^0 \longrightarrow C + \bar{C} + C^0$, then $H + \bar{H} \longrightarrow C + \bar{C}$ and $H^0 \longrightarrow C^0$. For if E^0 is an invariant cell in $F(H)$,[6] it is also in $F(\bar{H})$, hence fails to appear in $F(H + \bar{H})$. Therefore, the only invariant cells in $F(H + \bar{H} + H^0)$ are those of $F(H^0)$. Since $C + \bar{C}$ contains no invariant cells, we have $F(H^0) = C^0$.

2.2. If the chain $\Gamma + \bar{\Gamma} + \Gamma^0$ is a cycle, then $\Gamma + \bar{\Gamma}$ and Γ^0 are cycles. For $\Gamma + \bar{\Gamma} + \Gamma^0 \longrightarrow 0$ and by 2.1, $\Gamma^0 \longrightarrow 0$.

If an invariant cycle Γ is the boundary of an invariant chain, we shall write $\Gamma \simeq 0$; these "strong homologies" obey the same formal laws as ordinary homologies.

2.3. If Γ is a bounding cycle, then $\Gamma + \bar{\Gamma} \simeq 0$.

2.4. If $C + \bar{C} + C^0 \simeq 0$, then $C^0 \sim 0$ on K^0, and $C + \bar{C} \simeq 0$. For we have $H + \bar{H} + H^0 \longrightarrow C + \bar{C} + C^0$ and our statement follows from 2.1. In particular,

2.5. If $C + \bar{C} \simeq 0$, there is a bounding relation of the $H + \bar{H} \longrightarrow C + \bar{C}$.

2.6. If $C + \bar{C} \simeq 0$, then $F(C) \simeq 0$. For let $L = F(H) + C$; then $L \longrightarrow F(C)$ and $L + \bar{L} = F(H + \bar{H}) + C + \bar{C} = 0$. Hence, L is invariant and $F(C) \simeq 0$.

2.7. If D is a non-bounding cycle, and $D + \overline{D} \simeq 0$, then $D \sim \Delta \neq 0$, where Δ is invariant. For by 2.5, $H + \overline{H} \longrightarrow D + \overline{D}$. Let $\Delta = F(H) + D$. Then $\Delta \sim D$ and $\Delta + \overline{\Delta} = F(H + \overline{H}) + D + \overline{D} = 0$.

Returning to our base for p-cycles, we recall that the cycles D_p^i satisfy the relation $D_p^i + \overline{D}_p^i \sim 0$. It can happen that certain of the D's can be replaced by others which satisfy the stronger relation $D_p^i + \overline{D}_p^i \simeq 0$. We shall consider here only the case in which

A. $D_p^i + \overline{D}_p^i \simeq 0$ for every $p > 0$ and for every i.[8]

By 2.7 each D_p^i can now be replaced by an invariant cycle Δ_p, and our base now consists of the cycles Γ_p^i, $\overline{\Gamma}_p^i$, ${}^i\Delta_p (p = 1, \ldots, n)$.

Suppose ${}^i\Delta_p$ contains no cells of K^0. Then ${}^i\Delta_p = {}^iX_p + {}^i\overline{X}_p$ and $F({}^i\overline{X}_p) = {}^i\Delta_{p-1}^p$ is invariant, since $F(X) + \overline{F(X)} = F(\Delta) = 0$. Suppose again that ${}^i\Delta_{p-1}^p = {}^iX_{p-1}^p + {}^i\overline{X}_{p-1}^p$, and that ${}^i\Delta_{p-1}^p$ is not $\simeq 0$. Let $F({}^iX_{p-1}^p) = {}^i\Delta_{p-2}^p$ and thus continue, obtaining

$$(\Delta) \qquad {}^i\Delta_p^p (= {}^i\Delta_p), {}^i\Delta_{p-1}^p, \ldots {}^i\Delta_r^p \quad (r = r(p, i) \geq -1)$$

where, either ${}^i\Delta_r^p$ is the first cycle to contain cells of K^0, or the first to be $\simeq 0$. In the latter case ${}^i\Delta_r^p$ may be null—necessarily so, if $r = -1$.

2.8. *The cycles* $\Gamma_q^i + \overline{\Gamma}_q^i$, ${}^i\Delta_q^p$ $(q > 0)$ *in case A, are independent with respect to strong homology.*

PROOF.—For $q = n$, the statement is obvious. Assume it for $q + 1$, but suppose that for the dimension q the cycles in question satisfy a strong homology. Since this implies ordinary homology, and since ${}^i\Delta_q^p \sim 0$ for $q < p$, the cycles $\Gamma_q^i + \overline{\Gamma}_q^i$ and ${}^i\Delta_q^q$ cannot appear. Hence we have

$$H + \overline{H} + H^0 \longrightarrow x_{ip}{}^i\Delta_q^p \simeq 0 \quad (x_{ip} = 0 \text{ for } p = q).$$

Since $x_{ip}{}^iX_{q+1}^p \longrightarrow x_{ip}{}^iX_q^p$, $H + \overline{H} + H^0 + x_{ip}{}^i\Delta_{q+1}^p$ is a $(q + 1)$-cycle, say U and

$$U \sim y_i\Gamma_{p+1}^i + \bar{y}_i\overline{\Gamma}_{p+1}^i + z_i{}^i\Delta_{p+1}, \text{ say, and}$$

$$U + \overline{U} \simeq (y_i + \bar{y}_i)(\overline{\Gamma}_{p+1}^i + \overline{\Gamma}_{p+1}) \text{ by 2.3.}$$

But $U + \overline{U} = x_{ip}({}^iX_{q+1}^p + {}^i\overline{X}_{q+1}^p) = x_{ip}{}^i\Delta_{q+1}$. Thus there exists a strong homology for $q + 1$, which contradicts the hypothesis of the induction.

2.9. Corollary. *If* $r(p, i) \geq 0$, *then* ${}^i\Delta_r^p$ *contains cells of* K^0. For otherwise ${}^i\Delta_r^p \simeq 0$.

By the corollary we can write

$${}^i\Delta_r^p = {}^iX_r^p + {}^i\overline{X}_r^p + {}^iX_r^{0p} \text{ (for } r \geq 0)$$

where by 2.2, ${}^iX_r^{0p}$ is a cycle. The determination of the exact orders of minimal bases depends on a further study of the cycles ${}^iX_r^{0p}$. We shall examine only the special case in which

B. The cycles ${}^iX_r^{0p}$ are independent with respect to ordinary homologies on K^0.

2.10. *In the case AB, every cycle of the form* $C_q + \bar{C}_q$ $(q > 0)$ *is strongly homologous to a sum of cycles of the set* $\Gamma_q^i + \bar{\Gamma}_q^i + {}^i\Delta_q^p$.

PROOF. In any case we can write

$$C_q + \bar{C}_q \sim x_i\Gamma_q^i + \bar{x}_i\bar{\Gamma}_q^i + y_i\Delta_q^i.$$

On taking the transform of both sides and adding, we obtain $0 \sim (x_i + \bar{x}_i)(\Gamma_q^i + \bar{\Gamma}_q^i)$. Hence $x^i = \bar{x}_i$, and

$$(H_q) \qquad C_q + \bar{C}_q \sim x_i(\Gamma_q^i + \bar{\Gamma}_q^i) + y_i\Delta_q^i.$$

Since n is the dimension of our space, we can replace (H_n) by an equality, hence by a strong homology, proving the theorem for n. Assume it for $q + 1 \leq n$. We have

$$L_{q+1} \longrightarrow C_q + \bar{C}_q + x_i(\Gamma_q^i + \bar{\Gamma}_q^i) + y_i\Delta_q^i.$$

Hence $L_{q+1} + \bar{L}_{q+1}$ is a $(q+1)$-cycle, and by assumption,

$$L + \bar{L} \simeq z_i(\Gamma_{q+1}^i + \bar{\Gamma}_{q+1}^i) + u_{ip}\,{}^i\Delta_{q+1}^p. \qquad (1)$$

The last term can contain no Δ's for which $r(p, i) = q + 1$ otherwise (1) would be of the form

$$Y + \bar{Y} + x_{ip}\,{}^i\Delta_r^p \simeq 0 \ (x\text{'s not all } 0)$$

which in turn can be written

$$Z + \bar{Z} + x_{ip}\,{}^iX_r^{0p} \simeq 0.$$

Hence by 2.4, $x_{ip}\,{}^iX_r^{0p} \sim 0$ on K^0, which contradicts B. It follows that (1) is of the form

$$L + \bar{L} \simeq z_i(\Gamma_{q+1}^i + \bar{\Gamma}_{q+1}^i) + w_{ip}({}^iX_{q+1}^p + {}^i\bar{X}_{q+1}^p)$$

and by 2.6

$$F(L) \simeq w_{ip}F({}^iX_{q+1}^p); \text{ that is}$$

$$C_q + \bar{C}_q + x_i(\Gamma_q^i + \bar{\Gamma}_q^i) + y_i\,{}^i\Delta_q^q \simeq w_{ip}\Delta_q^p$$

which completes the proof.

3. Let $v_i = (V_i\bar{V}_i) = (\bar{V}_iV_i)$, where $V_1, V_2, \ldots$ are the vertices of K. Suppose $E_p = V^{i0}, \ldots, V^{ip}$; then no two vertices of E_p are transforms of each otherwise they would determine an invariant 1-cell which is not pointwise invariant. Hence $v^{i0}, \ldots, v^{ip}$ are distinct and can therefore be thought of as determining a non-singular p-simplex, ΛE_p. If E_q is a face of E_p, then ΛE_q is a face of ΛE_p and hence the totality of cells ΛE is a connected complex k_n; and the subcomplex k^0 obtained from K^0 is identical

in structure with K^0. Let $\Lambda(x_iE_p^i)$ mean the chain $x_i(\Lambda E_p^i)$; then clearly $\Lambda F(E_p^i) = F(\Lambda E_p^i)$.

Let $e_p = \Lambda E_p$. We denote the chain $E_p + \bar{E}_p$ by $\Lambda' e_p$ and define $\Lambda'(x_i e_p^i) = x_i(\Lambda' e_p^i)$. Since $\Lambda F(E_p) = F(e_p)$, we have

$$\Lambda' F(e_p) = F(E_p) + \overline{F(E_p)} = F(E_p + \bar{E}_p); \text{ that is,}$$

$$\Lambda' F(e_p) = F(\Lambda' E_p).$$

Thus, boundary relations are preserved under Λ and Λ'.[9] Hence cycles transform into cycles and

3.1. If $\Gamma \sim 0$, then $\Lambda\Gamma \sim 0$.

3.2. If $\gamma \sim 0$, then $\Gamma'\gamma \simeq 0$.

3.3. Λ determines a homeomorphism between K^0 and k^0.

3.4. If c is a cycle in k, $\Lambda' c$ can be written in the form $C + \bar{C}$, where $\Lambda C = c$. For let $c = x_i e_p^i + y_j e_p^{0j}$, where $e_p^i = \Lambda E_p^i = \Lambda \bar{E}_p^i$, $e_p^{0j} = \Lambda E_p^{0j}$. We can take for C the chain $x_i E_p^i + y_j E_p^{0j}$.

3.5. Suppose $C + \bar{C} \simeq 0$. If ΛC is a cycle, then $\Lambda C \sim$ a cycle on k^0; if ΛC is not a cycle, then its boundary is in k^0, and is ~ 0 in k^0. For by 2.5, $H + \bar{H} \longrightarrow C + \bar{C}$. If $H \longrightarrow C + X$, then $X + \bar{X} = 0$ and $X = X' + \bar{X}' + X^0$. Hence $\Lambda H \longrightarrow \Lambda C + \Lambda X^0$. Now if ΛC is a cycle, then so is ΛX^0, and $\Lambda C \sim \Lambda X^0$. In any case $\Lambda C + \Lambda X^0 \longrightarrow 0$; hence $F(\Lambda C) = F(X^0) \sim 0$ on K^0.

We now seek a base for q-cycles in k. Note that if $q \geq r(p, i) + 2$, then $\Lambda({}^i\Delta_{q-1}^p) = \Lambda({}^iX_{q-1}^p + {}^i\bar{X}_{q-1}^p) = 0$; hence

$$\Lambda({}^iX_q^p) \longrightarrow \Lambda F({}^iX_q^p) = \Lambda({}^i\Delta_{q-1}^p) = 0.$$

Thus for $q \geq r(p, i) + 2$, $\Lambda({}^iX_q^p)$ is a cycle, say ${}^i\xi_q^p$. Let $\Lambda(\Gamma_q^i) = \gamma_q^i$ ($q = 1, \ldots, n$). We have

$$\Lambda'({}^i\xi_q^p) = {}^iX_q^p + {}^i\bar{X}_q^p = {}^i\Delta_q^p; \quad \Lambda'\gamma_q^i = \Gamma_q^i + \bar{\Gamma}_q^i. \tag{2}$$

3.6. *The cycles ${}^i\xi_q^p$ and γ_q^i* (in case A) *are independent with respect to homologies in k.* For by 3.2 and (2) a homology $x_{ip}\,{}^i\xi_q^p + y_i\gamma_q^i \sim 0$ would imply $x_{ip}\,{}^i\Delta_q^p + y_i{}^i\Gamma_q^p \simeq 0$, contradicting 2.8.

3.7. *In the case AB, every q-cycle ($q > 0$) in k is homologous to a sum of cycles ${}^i\xi_q^p$, γ_q^i, plus a cycle in k^0.*

PROOF. Let γ be a q-cycle in k, and let $\Lambda'\gamma = \Gamma + \bar{\Gamma}$ where $\Lambda\Gamma = \gamma$ (3.4). By 2.10,

$$(H) \qquad \Gamma + \bar{\Gamma} \simeq x_i(\Gamma_q^i + \bar{\Gamma}_q^i) + y_{ip}\,{}^i\Delta_q^p.$$

Now $y_{ip} = 0$ whenever $r(p, i) = q$; otherwise (H) is of the form

$$Y + \bar{Y} + z_{ip}({}^iX_q^p + {}^i\bar{X}_q^p + {}^iX_q^{0p}) \simeq 0 \quad (z\text{'s not all } 0)$$

which by 2.4 implies $z_{ip}\,{}^iX_q^{0p} \sim 0$ on K^0, contradicting B. Moreover $y_{ip} = 0$ whenever $r(p, i) = q + 1$; otherwise (H) is of the form

$$Z + \bar{Z} + \Sigma u_{ip}({}^iX_q^p + {}^i\bar{X}_q^p) \simeq 0 \quad (u\text{'s not all } 0)$$

where ΛZ is a cycle and p, i satisfy the relation $r(p, i) + 1 = q$, so that $\Lambda({}^iX_q^p) \longrightarrow \Lambda({}^iX_{r(p,i)}^{0p}) = {}^ix_r^{0p}$, say. Now $\Sigma u_{ip}{}^ix_r^{0p}$ being the boundary of $\Lambda(Z + \Sigma u_{ip}{}^iX_q^p)$ is ~ 0 on k^0 by 3.5, and this implies $\Sigma u_{ip}{}^iX_{q-1}^{0p} \sim 0$ on K^0 by 3.3, which contradicts B. It now follows that (H) can be written

$$\Gamma + \bar{\Gamma} + x_i(\Gamma_q^i + \bar{\Gamma}_q^i) + \Sigma({}_iX_q^p + {}^i\bar{X}_q^p) \simeq 0$$

where the q-cycles ${}^iX_q^p + {}^i\bar{X}_q^p$ are such that $\Lambda({}^iX_q^p) = {}^i\xi_q^p$. Hence by 3.5

$$\gamma + x_i{}^i\gamma_q^p + \Sigma^i\xi_q^p \sim \xi^0, \text{ a cycle on } k^0.$$

3.8. *If the cycles ${}^iX_q^{0p}$ $(q = r(p, i))$ are a base for q-cycles for with respect to homologies in K^0, then for the case AB the cycles γ and ξ of* 3.7 *are a minimal base for q-cycles of k.* For by 3.1 the cycles ${}^ix_q^{0p}$ are a base with respect to homologies in k^0. But ${}^ix_q^{0p} = F(\Lambda({}^iX_{q+1}^p))$; hence ${}^ix_q^{0p} \sim 0$ on k, and the same holds for every q-cycle of k^0; 3.8 then follows from 3.7.

Under the hypotheses of 3.8 we can evaluate the mod 2 Betti numbers of k as follows: let R_q^Γ be the number of cycles Γ_q^i and let S_q be the number of distinct pairs p, i satisfying the inequality $r(p, i) + 2 \leq q$. Then

3.9. $$R_q(k) = R_q^\Gamma(k) + S_q(k),^{10} \; q = (1, \ldots, n).$$

EXAMPLE. Let T be an orthogonal involution of an n-sphere H_n. Then it is easy to obtain a cellular subdivision satisfying (a), (b). Now K^0 is an H_s $(-1 \leq s \leq n)$; $R_q^\Gamma = 0$ for $q > 0$, and the only cycle of type Δ is ${}^1\Delta_n^n = H_n$. Moreover $r(n, 1) = s$; in fact it is easy to see that the sequence (Δ) can be so constructed that ${}^1\Delta_r^n = H_s$; hence A, B and the hypothesis of 3.8 are satisfied. For $q \geq s + 2$, the inequality $r(p, i) + 2 \leq q$ has just one solution, namely $(n, 1)$. Hence by 3.9, $R(k) = 1$ for $q = s + 2, \ldots, n$, while the remaining values are 0 (except $R_0(k) = 1$). In particular, if T is a reflection across the center of H_n, then $s = -1$ and $R_q(k) = 1$ for each q; k is here homeomorphic with an n-dimensional projective space.

4. Symmetric products. Let $K_{2n} = L_n \times L_n'$ where L and L' are of identical structure and let p, p' be corresponding points of L, L'. Consider the involution

$$T(p \times q') = q \times p'.$$

There is no difficulty[11] in obtaining a subdivision of K_{2n} of the type considered. The complex k_{2n} is the "symmetric product" of L by itself.

The q-cycles of a minimal base in K_{2n} are $a_h^i \times a_{q-h}^{\prime j}$, where the a_h's are a minimal base for h-cycles in L.[12] Hence the bases for K_{2n} are of the (Γ, Δ)-type considered. The Δ's occur only in even dimensions—${}^i\Delta_{2h} = a_h^i \times a_h^{\prime i}$. Now K^0 consists of all points $p \times p'$ and is therefore homeomorphic with L. Moreover each ${}^i\Delta_{2h}$ contains in the same way a sub-

complex ${}^{i}\Delta_h^0$ homeomorphic with a_h^i, and the cycles ${}^{i}\Delta_h^0$ are a minimal base for h-cycles in k^0. It can be shown that $r(2h, i) = h$; in fact, the construction of the sequence (Δ) can be carried out in such a way that ${}^{i}\Delta_r^{2h} = X_h^i + \overline{X}_h^i + {}^{i}\Delta_h^0$. Hence A, B and the hypothesis of 3.8 are satisfied, and application of 3.9 yields

$$R_q(k_{2n}) = \begin{cases} R_q^\Gamma(K_{2n}) \text{ for } q = 1, 2. \\ R_q^\Gamma(K_{2n}) + R_{2t}^\Delta(K_{2n}) + R_{2(t+1)}^\Delta(K_{2n}) + \ldots + R_{2(q-2)}^\Delta(K_{2n}), (q > 2) \end{cases}$$

where $t = \left[\dfrac{q+1}{2}\right]$ and R_{2s}^Δ means the number of cycles ${}^{i}\Delta_{2s}$ of K_{2n}; $R_{2s}^\Delta = 0$ for $s > n$.

The various R's of K_{2n} can be computed in terms of the Betti numbers of L_n. If, for example, L_n is a sphere H_n, then $R_n^\Gamma(K_{2n}) = R_{2n}^\Delta(K_{2n}) = 1$, and all other R's of dimension > 0 are 0. Hence

$$R_0(k_{2n}) = 1, R_q(k_{2n}) = 0 \text{ for } q = 1, 2, \ldots, n-1 \text{ and } q = n+1;$$
$$R_q(k_{2n}) = 1, \text{ for } q = n \text{ and } q = n+2, \ldots, 2n.$$

[1] Not yet published.

[2] For notation, definitions, etc., see Lefschetz, "Topology," *Amer. Math. Soc. Colloquim Publications*, **12**, New York (1930).

[3] To what extent these conditions restrict the problem will be considered elsewhere; we are here considering only its combinatorial aspects.

[4] A repeated index means summation over all its allowable values.

[5] $F(X)$ means the boundary of X.

[6] E "is in the chain X" if it appears in the symbol for X with a non-zero coefficient.

[7] Null cycles will be considered as being invariant; hence $X = Y$ implies $X \simeq Y$.

[8] For $p = 0$ this assumption would rule out transformations which leave no points invariant.

[9] The correspondences analogous to Λ and Λ' for the non-modular case are used by Mr. Richardson.

[10] All Betti numbers are, of course, mod 2; we omit the 2 in our symbols.

[11] Since T is linear in patches.

[12] Lefschetz, loc. cit., p. 228.

ON SUPRACONDUCTIVITY AND THE HALL EFFECT

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I venture to raise two questions, each perhaps heretical, concerning metals in the supraconductive state. The first relates to the Hall effect; the second will be stated somewhat later.

Is the experimental evidence from which Ounes inferred the non-existence of the Hall effect in the supraconductive state of metals satisfactory?

Supraconduction shows itself in two ways. The first way, the one by which the phenomenon was discovered in solid mercury, is that of a vanishingly small difference of potential between two points a considerable distance apart on a piece of metal forming part of a circuit through which a current is maintained by an applied e. m. f., the circuit as a whole not being supraconductive. The second, and even more astonishing, way is by the magnetic moment of currents, set up within the metal by induction, which persist for hours, apparently without diminution, after the inducing influence has ceased to act. Ounes used both of these manifestations of supraconduction in his test for the Hall effect, the metal used being in each case lead.

In his first test, by what may be called the direct method, he used the ordinary arrangement for observing the Hall effect; i.e., placing the plate of metal, carrying an electric current, between the poles of an electromagnet and looking for a transverse difference of potential due to the action of the magnetic field. He worked at two temperatures, 4.°25K. and 2.°8K., each well below the critical temperature of supraconductivity in lead. Using a magnetic field too weak to destroy supraconduction, he found merely that the Hall coefficient, R, was at the higher temperature less than 2×10^{-5}, and at the lower temperature less than 6×10^{-5}. By using a sufficiently powerful magnetic field to abolish the supraconductive state he found a measurable Hall effect, R being 18×10^{-5} at 4.°25K. and 13×10^{-5} at 2.°8K. The most, then, that can be claimed for this test, even if the logic of the method employed is accepted, is that it shows the value of the Hall effect in the supraconductive state of lead to be several times smaller than the value in the normal conductive state.

The second method used by Ounes in looking for a Hall effect in the supraconductive state may be described as follows: A hollow sphere of lead in the supraconductive state was suspended by a torsion spring in a horizontal magnetic field and by variation of the intensity of this field a system of persistent currents was set up within the lead, the plane of

these currents being perpendicular to the direction of the magnetic field. By twisting the suspending spring the sphere was turned so that the axis of these currents made an angle, in the horizontal plane, with the direction of the imposed magnetic field. This angle, the amount of torsion in the suspension being undiminished, remained constant, so far as could be seen, for several hours.

Ounes interpreted this experiment as showing that in supraconductive lead there is no Hall effect, no tendency for an electric current to move sidewise through the metal under the pull of the ponderomotive force of the magnetic field, whereas in the normal conductive state of a metal there is, apparently, such a tendency. This conclusion is paradoxical, for it seems to imply that electric currents have in one respect less freedom of motion in the supraconductive state than in the normal state. Accordingly Ounes was led to conceive of the persistent currents as flowing in channels or tubes, the walls of which precluded transverse motion, though the idea of such tubes as existing within a supraconductive metal was no less paradoxical, perhaps, than the phenomenon it was invoked to explain. In this view of the matter he had the support of Lorentz.

The logic of both these tests made by Ounes seems to be open to serious question, for in both cases it is, apparently, assumed that the lines of induction of an external magnetic field can penetrate fully metals in the supraconductive state. We know that in any ordinary good conductor penetration by magnetic flux is sensibly resisted and retarded by the induced Foucault currents which the changing state of induction sets up within the metal. The greater the conductivity, the greater is this resistance to or retardation of the magnetic penetration. Should we not, then, expect this penetration to be very slow, perhaps indefinitely retarded, in the supraconductive state?

Clerk Maxwell in Art. 654 of his "Electricity and Magnetism," 2nd edition (1881), begins a discussion "on the induction of electric currents in a sheet of infinite conductivity," and in Art. 655 he says, "The sheet may therefore be regarded as impervious to magnetic induction, and the lines of magnetic induction will be deflected by the sheet exactly in the same way as the lines of an electric current in an infinite and uniform conducting mass would be deflected by the introduction of a sheet of the same form made of a substance of infinite resistance. If the sheet forms a closed or an infinite surface, no magnetic actions which may take place on one side of the sheet will produce any magnetic effect on the other side."

This statement of Maxwell's seems to apply both to the flat sheet of lead used by Ounes in his first Hall effect test and to the leaden shell used in his second test. To be sure, we are not justified in assuming for actual metals in the supraconducting state a really infinite conductivity such as Maxwell assumes for his imagined sheet or surface. The currents which

are set up by induction within, or "on the surface of," real metal in the supraconductive state would not be set up if no change whatever occurred in the amount of magnetic flux within the material. But, as we cannot at present put any definite limit to supraconductivity, we cannot put any definite limit to the slowness with which lines of magnetic induction would penetrate supraconductive metal. If the conductivity of the metal is great enough to allow currents, once started, to persist for hours without perceptible diminution, may it not be great enough to prevent for an equal time perceptible penetration of the metal by magnetic flux?

We have had, then, I think, no satisfactory test for the existence of a Hall effect in the supraconductive state of metals. But apparently such a test could be made. Set up the Hall effect in a metal at a temperature just above the conductive critical temperature and note whether the effect continues when the metal is cooled below this temperature, the magnetic field continuing in force.

Such an experiment would be of considerable theoretical interest. If the Hall effect is immediately dependent on the longitudinal potential-gradient within the metal in the magnetic field, because of some slight distortion produced by this field, the effect should vanish when this potential-gradient vanishes, as it does in the supraconductive state. If, on the other hand, the effect is immediately dependent on the intensity of the longitudinal current, and so on the magnitude of the ponderomotive force exerted on the current by the magnetic field, we should expect it to persist in the supraconductive state.

It may be well to remark here that neither the existence of a ponderomotive force acting on a supraconductor carrying a current through a magnetic field nor such a torque as Ounes found with his leaden shell subjected to in a magnetic field requires us to suppose penetration of the conducting material by the lines of magnetic induction.

According to my conception of a dual electric conduction, only a fraction of the total normal conduction being attributed to the free electrons, the net Hall effect is the resultant of highly complicated conditions and actions in the normal state of metals. I am, however, of the opinion that supraconduction is a much simpler process than ordinary conduction, being effected by means of electrons permanently free so long as the supraconductive state continues. I think that in all probability a Hall effect exists in supraconducting metals and I should not be much surprised to find it of the same sign in all metals in the supraconductive states. I have no confident expectation, however, as to this matter of sign. We know that electrons moving in free space through a magnetic field must drift in the direction of the ponderomotive force exerted on them by this field. But to assume from this that free electrons moving between the atoms of a metal must drift in the direction of the magnetic ponderomotive

force acting upon them seems to me a fallacy. Any object merely floating in the air must move in the direction of the wind, but a boat under the action of wind and the reaction of water does not necessarily move in the direction of the wind.

I come now to my second heretical question. Is there conclusive evidence that the persistent currents which Ounes and others have observed are anything more than the aggregate of microscopic electric whirls within the metal? Is there conclusive evidence that the persistent current which is ordinarily assumed to be circumferential within a supraconductive ring or shell is really circumferential?

As bearing on this question I will quote two passages from the 1924 Solvay Conference paper of Ounes. On p. 251 of the Conference Report he speaks of an experiment of Mr. Langevin "où une bobine à circuit ouvert montrait un courant persistant." On pp. 263 and 264 he describes an experiment of his own made with a ring consisting of 24 alternative sectors of tin and lead. These metal sectors, which were soldered together, were thin layers covering a ring of ivory, the junctions between them being on radii of the ring. "Le courant [persistent] fut établi avec un champ perpendiculaire au plan de l'anneau, puis celui-ci fut tourné d'un angle de 30°. Nous avions pensé que nous trouverions un courant qui s'étendrait au bout d'un certain temps [because the soldered junctions were not supposed to be supraconductive] mais l'expérience a montré que des courants continuaient à circuler dans l'anneau et, lorsque l'expérience fut répéteé avec l'anneau coupé, celui-ci montra le même moment magnétique."

A chain being no stronger than its weakest link, it seems probable that local currents of very limited radius would be more likely to persist than currents having a long cyclic path. Currents of the latter description may well be induced in a supraconductor when the original penetrating magnetic field is varied but they are likely to die out sooner than the local whirls of current. Is it not reasonable to suppose that we have here an explanation of the fact noted by Ounes, on p. 255 of the Solvay Report, that just after a change of the imposed magnetic field the induced current "varie encore un peu?"

I must, of course, speak very cautiously of this matter, for I have never even seen an experiment on supraconductivity. It seems to me, however, that, for example, the conclusions reached by McLennan[1] and his coworkers as to the existence and strength of circumferential persistent currents in small rings of tantalum, lead and tin, respectively, are open to question. These investigators make no mention of the possibility that the currents are not circumferential. They assume them to be circumferential and on this assumption estimate their strength, from the observed magnetic torque between each supraconductive ring and a neighboring coil of copper wire carrying a current. I believe, however, that all of the

phenomena they describe are quite consistent with the supposition that the persistent currents were local whirls within the metal rings, not circumferential currents at all.

Apparently a test of the question here raised could be made by determining the direction and intensity of the magnetic field along an axis common to the ring and the surrounding coil of current-bearing wire. The investigators assumed, I believe, that the magnetic flux along the axis was zero after the persistent current in the ring was established. If my idea of the matter is correct, there should be along this axis a permanent flux corresponding to the direction of the current circulating in the copper coil.

[1] *Phil. Mag.*, 168–180, July (1932).

THE KATABOLISM OF THE NON-VOLATILE ORGANIC ACIDS OF TOBACCO LEAVES DURING CURING[1]

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In a recent study[2] of the changes that take place in the non-volatile organic acids during the process of curing of the leaves of Connecticut shade-grown tobacco, we showed that the proportion of malic acid altered very little, but that the proportion of citric acid increased materially. In those experiments, however, an unsatisfactorily low proportion of the total organic acidity of the leaves was recovered in the form of identified organic acids and only traces of oxalic acid were detected. Further study of the methods of analysis previously employed has now revealed several unsuspected sources of error and a revision of these methods is to be presented in a forthcoming publication from this Station.[3]

The newer modifications of these methods have been employed in a further study of the metabolism of the non-volatile organic acids of tobacco leaves during curing. The material employed consisted of three 50 kilo lots of leaves (8th to 11th leaf) picked the same day (August 1, 1929). One lot was immediately extracted with boiling water, the other two were cured for 12 and for 51 days, respectively, before extraction. The polybasic organic acids were precipitated as barium salts from the concentrated hot water extracts of the tissue by the addition of two volumes of alcohol. The barium salts were decomposed with sulphuric acid, the liberated acids were extracted by ether, esterified and the esters distilled, the individual acids being identified as hydrazides or other suitable derivatives.

By far the greater part of the distilled esters consisted of diethyl malate. Triethyl citrate made up a large part of the remainder and these two esters alone accounted for approximately 90 per cent of the esters derived from the partially and fully cured leaves.

Indirect determinations of malic acid by the polarimetric method of Dunbar and Bacon[4] and of citric acid by the pentabromoacetone method of Hartman and Hillig[5] were likewise carried out. The results were closely checked by the results of the ester distillation and showed that these two indirect methods are thoroughly trustworthy for the analysis of tobacco leaf.

Oxalic acid presented an entirely special problem. Although we had reason to believe that tobacco leaves of the type employed contain considerable quantities of oxalic acid, traces only of diethyl oxalate were found among the distilled esters. This drew our attention to the unsuspected volatility of oxalic acid. It was found that large losses occur during the dehydration with alcohol and the esterification procedures as usually conducted, and also that losses frequently amounting to 100 per cent of the oxalic acid present were experienced when aqueous solutions were repeatedly concentrated to a sirup in vacuo. It was therefore necessary to employ a method in which concentrations of aqueous solutions were avoided. Accordingly, the oxalic acid was extracted with ether from the powdered tissue, previously adjusted to pH 1 with dilute sulphuric acid, and determined as calcium oxalate. This procedure was shown by independent experiments to yield reliable results.

In table 1 are shown the results of the analysis of the three samples of tobacco leaves mentioned above. The figures are gram equivalents

TABLE 1

THE ORGANIC ACIDS OF THE FRESH LEAVES, THE 12-DAY CURED AND THE FULLY CURED LEAVES OF TOBACCO EXPRESSED AS GRAM EQUIVALENTS PER 50 KILOS OF ORIGINAL FRESH WEIGHT

	FRESH LEAF	12-DAY CURED	FULLY CURED
Total organic acidity	14.6	14.3	13.6
Organic acids of water extract	10.6	11.1	10.6
Oxalic acid	2.92	2.72	2.66
Malic acid	5.84	6.03	4.82
Citric acid	0.62	2.99	3.60
Unknown polybasic acids	2.54	0.78	0.58
Unknown monobasic acids	1.6	1.3	1.6
Percentage of total organic acidity in form of known acids	64.1	82.1	81.5

in a quantity of the tissue that represented 50 kilos of original fresh weight. The total organic acidity was determined by electrometric titration between the limits pH 7.8 and 2.6 of the solution prepared for the determi-

nation of oxalic acid. The organic acids of the water extract were determined upon a solution prepared by ether extraction of an acidified hot water extract of the leaves. A part of the difference between the results is due to the fact that oxalic acid is present as insoluble calcium oxalate in the tissue and, consequently, remains in the residues from the extraction. The malic and citric acid were determined by the methods of Dunbar and Bacon, and of Hartman and Hillig, respectively, in the solution obtained from the barium salts precipitate. The unknown polybasic acids represent that part of the acidity in this solution that was not accounted for as malic and citric acid. The figures include the small proportions of succinic and fumaric acids found during distillation of the esters. The figures for unknown monobasic acids represent that part of the acidity of the water extract of the tissue that was not precipitated as barium salts by two volumes of alcohol; it is assumed that this acidity is largely due to monobasic acids since previous experiments[6] have shown that all the polybasic acids commonly found in leaves are quantitatively precipitated under these conditions.

Oxalic acid remains substantially unchanged during the process of curing tobacco leaves and the quantity of malic acid is reduced to only a small extent; the katabolic processes that occur in the leaves during curing affect these two acids but little. There is also little or no change in the part of the acidity we assume to belong to monobasic acids. The quantity of citric acid is enormously increased; even in the earliest stage of the curing process the amount of citric acid becomes five times greater than that present in the fresh leaves, and synthesis of citric acid apparently continues, though at a lower rate, throughout the curing period. The amount of unknown polybasic acids decreases almost as precipitately as the citric acid increases, and the quantities involved are such as to lend some support to the view that a part, at least, of the newly formed citric acid has its origin in these unknown acids. A study of the soluble carbohydrate content of these same samples of leaves also suggested that some of the citric acid may have arisen as a result of carbohydrate metabolism. The total acidity of the leaf samples changed very little if at all in spite of the extensive change in the citric acid and the simplification of the organic acid picture that occurred.

The present data indicate that the results of analyses of cured tobacco for oxalic, citric and malic acids, provided methods of demonstrated reliability are employed, will furnish unusually complete information regarding the organic acids present. It is hoped that the investigations now going forward in this laboratory will yield qualitative information at least regarding the still unknown part of the total acidity.

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] Vickery, H. B., and Pucher, G. W., *J. Biol. Chem.*, **90**, 637 (1931).

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THE ENERGY OF UREA SYNTHESIS

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This study of the energy changes accompanying the synthesis of urea by living tissue from ammonia and carbon dioxide was undertaken in order to obtain more information regarding the causes of the specific dynamic action of protein; and because this reaction seems to be particularly suitable for the study of the energy changes in biological coupled reactions. In the synthesis of urea from ammonia and carbon dioxide (in which a large fraction of the ammonia is converted) there is a gain in free energy, which can be derived only from some other free energy liberating reaction.

Krebs and Henseleit[1] have shown that measurable quantities of urea are synthesized by liver slices prepared according to the method of Warburg.[2] Employing essentially their method we have confirmed this observation as well as their finding that a considerably greater rate of synthesis is obtained when ornithine is added to the fluid in which the slices are immersed.

Our immediate objective was a reëxamination of the relationship between oxygen consumption and urea synthesis. Krebs and Henseleit were unable to find, within the limits of their experimental error, any increase in oxygen consumption after ornithine was added to lactate Ringer's solution containing ammonia, even though the amount of urea synthesized was increased several fold. In their protocoll bearing on this question the rates of O_2 consumption (given as Q_{O_2}) were 20.5 and 23.9 in the experiment where the rate of urea synthesis (Q_{urea}) was 3.19, and were 21.1 and 20.4, where in the presence of ornithine, the Q_{urea} was 11.83. Because of these rather large variations in the rates of oxygen consumption we employed several modifications of their technique. These were the use of much larger amounts of liver tissue, quantities

varying from 20 to 50 mg. in each experimental vessel to reduce sampling errors; and further, each liver slice was cut into two approximately equal pieces, one being put into the control, the other into the experimental vessel. For the measurement of the oxygen consumption Krebs and Henseleit employed the Warburg two-vessel method.[3] In our experiments the CO_2 was absorbed by NaOH so that the O_2 consumption could be observed continuously, and the experiment terminated soon after the rate of respiration in either experimental or control vessel began to decline. The data given in table 1 show that though the concordance between duplicates was not as good in lactate as in glucose Ringer's solution, nevertheless in both types of solution the difference between duplicates was less than in the typical protocoll given by Krebs and Henseleit.

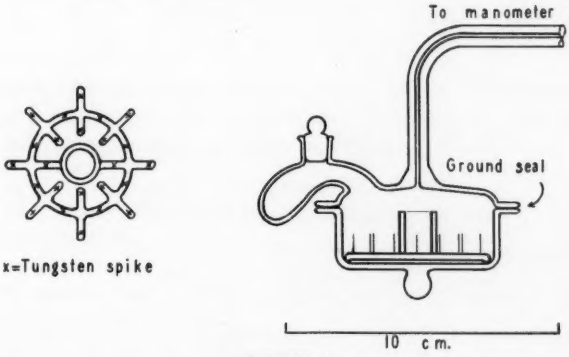

FIGURE 1

Diagram of manometer vessels and glass ring carrying the tungsten spikes on which tissue slices are mounted.

For these large quantities of tissue dessicator-shaped vessels were used, about 6.5 cm. in diameter, 3 cm. high and 74 cc. in volume. Their mode of attachment to the manometer and the seal are depicted in figure 1. The surfaces of the ground glass flanges constituting the seal were coated with lanolin-vaseline grease; and after the two parts of the vessel were put together, paraffin (m. p. 51–53°C.) was painted around the seal to make it gas tight. Elastic rubber bands passing under the lower part, and held by metal hooks on a removable metal ring on the upper part, took the strain off the paraffin, and helped to hold the seal tight.

The slices were carried on thin tungsten spikes fused into a glass ring which fitted snugly in the bottom of the vessel so that there was no sliding when the vessel was rocked. The glass ring was fused to a central well for the NaOH, the spikes and well being removable as one piece. We found this device more convenient, when many slices are to be used,

than glass spikes fused to the bottom of the vessel. Tungsten spikes, as far as we could detect, are not more injurious to liver slices than glass.

TABLE 1

RATES OF RESPIRATION OF SIMILAR LIVER SLICES IN TWO DIFFERENT VESSELS

TYPE OF RINGER'S SOLUTION	DURATION OF EXPT. MIN.	No. 1 WEIGHT OF TISSUE MG.	No. 1 Q_{O_2}	No. 2 WEIGHT OF TISSUE MG.	No. 2 Q_{O_2}
Glucose	50	31.4	8.67	30.4	9.05
"	20	24.4	8.66	25.8	8.86
"	60	36.6	8.74	36.6	8.83
"	25	36.2	8.80	35.0	9.20
"	15	57.2	8.91	55.0	9.36
"	40	61.4	10.57	64.2	11.15
Lactate	65	29.4	10.96	28.9	12.08
"	45	33.2	12.17	30.0	13.06
"	30	43.4	10.12	36.4	10.85

The composition of the Ringer's solution was that employed by Krebs and Henseleit; but ammonium bicarbonate was used instead of ammonium chloride as a source of ammonia. The gas was tank oxygen, and the

TABLE 2

UREA SYNTHESIS IN GLUCOSE RINGER'S SOLUTION

EXPT. NO.	DRY WEIGHT OF LIVER USED MG.	ORNITHINE CONC. MG. PER CENT	NH_4 CONC. MG. PER CENT	DURATION OF UREA SYNTHESIS MINUTES	Q_{O_2}	Q_{urea}	ΔQ_{O_2}	ΔQ_{urea}	$\frac{\Delta Q_{O_2}}{\Delta Q_{urea}}$
1	31.4	0	0	90	7.52	0.26	1.49	1.55	1.0
	30.4	0	25		9.01	1.81			
2	24.4	0	0	105	9.36	1.13	0.31	0.51	0.6
	25.8	0	25		9.67	1.84			
3	50.2	0	0	120	8.06	0.79	1.89	2.08	0.9
	46.6	0	25		9.95	2.87			
4	36.6	0	0	80	8.40	0.38	0.66	0.72	0.9
	36.6	0	25		9.06	1.10			
5	36.2	3.3	0	50	7.39	1.05	1.63	2.04	0.8
	35.0	3.3	25		9.02	3.09			
6	32.6	0	0	85	12.48	0.38	0.53	0.49	1.1
	32.8	0	50		13.01	0.87			
7	33.0	0	0	95	6.64	0	0.82	1.26	0.7
	31.6	0	50		7.46	1.26			
8	50.2	6.5	0	165	9.37	0.40	3.81	4.98	0.8
	49.5	6.5	50		13.18	5.38			
9	37.9	6.5	0	145	7.66	0.23	3.82	4.70	0.8
	39.4	6.5	50		11.48	4.93			

experiments were carried out at 37.4°C. The urea was determined by the method described by Krebs and Henseleit, with the modification of

the addition of a small amount of freshly prepared haemoglobin solution to accelerate the CO_2 equilibration.

The animals used were white rats. The liver slices were prepared according to the manner described by Warburg. Their average thickness was about 0.3 mm. In a typical experiment the slices were mounted on spikes, placed in 15 cc. Ringer's solution, to which was added 1 cc. of ammonia, ammonia-ornithin or Ringer's solution. The stock ammonia, and neutralized *d*-ornithine solutions were made up in Ringer's solution.

TABLE 3

UREA SYNTHESIS IN *d-l* LACTATE RINGER'S SOLUTION

EXPT. NO.	DRY WEIGHT OF LIVER USED MG.	ORNITHINE CONC. MG. PER CENT	NH_4 CONC. MG. PER CENT	DURATION OF UREA SYNTHESIS MINUTES	Q_{O_2}	Q_{urea}	ΔQ_{O_2}	ΔQ_{urea}	$\frac{\Delta Q_{O_2}}{\Delta Q_{urea}}$
10	29.4	0	0	105	9.80	0.30	1.69	1.17	1.4
	28.9	0	50		11.49	1.47			
11	33.2	6.5	0	80	10.82	0.94	2.64	4.29	0.6
	30.0	6.5	50		13.46	5.23			
12	34.2	0	0	110	12.52	0.33	3.49	1.82	1.9
	29.0	0	50		16.01	2.15			
13	19.6	0	0	100	13.68	0.16	1.65	1.46	1.1
	19.4	0	50		15.33	1.62			
14	34.6	6.5	0	95	9.09	0.39	2.92	3.32	0.9
	33.2	6.5	50		12.01	3.71			
15	27.2	6.5	0	140	11.23	0.25	2.59	3.44	0.8
	30.4	6.5	50		13.82	3.69			
16	18.0	6.5	0	90	9.96	0	2.94	3.38	0.9
	19.0	6.5	50		12.90	3.38			
17	28.0	6.5	0	90	10.79	0.20	4.76	4.40	1.1
	28.2	6.5	50		15.55	4.60			
20	31.0	0	47	100	12.45	2.34	2.91	3.97	0.7
	29.8	6.0	47		15.36	6.31			
22	19.2	0	50	130	14.27	1.37	2.64	2.75	1.0
	20.0	6.5	50		16.91	4.12			

0.5 cc. 10 per cent NaOH was added to the central well. For the urea determinations 10 cc. of solution were used. To this were added 1.5 cc. of acetate buffer and 0.3 cc. of freshly prepared 1 per cent human haemoglobin solution, with 1 cc. urease solution in the side arm.

With this technique we have found in every instance an increased oxygen consumption accompanying the synthesis of urea, in more than twenty-five experiments covering a variety of conditions (tables 2 and 3).

Each pair of figures shown in these tables was obtained from a different animal, there being only three vessels at our disposal for these experiments, of which one was necessarily the thermobarometer. The range of possible experiments was therefore restricted, and also the accuracy of the results.

Those obtained so far suggest that one molecule of oxygen is consumed for every molecule of urea which is formed, whether ornithine is added or not, and whether glucose or lactate Ringer's is used. For the present we are not inclined to attribute more than a qualitative significance to these results; and we shall postpone discussion of their bearing on the question of the energetics of the coupled reaction until after our experiments to obtain more concordant measurements, which are now in progress.

In spite of this qualification it seems justifiable to conclude even from these results that part of the specific dynamic action of protein must be ascribed to the superfluous energy released in the liver in the synthesis of urea from ammonia and carbon dioxide. The maximum contribution of this process is probably not more than 20 per cent of the total. This is in accord with the hypothesis proposed two years ago[4] that the increase in metabolism following the ingestion of protein is to be attributed to several processes, and that among these is the metabolism of the nitrogen.

TABLE 4

EFFECT OF ORNITHINE ALONE ON THE RATE OF RESPIRATION OF LIVER SLICES IN *d-l* LACTATE RINGER'S SOLUTION. ALL ANIMALS FROM SAME LITTER

EXPT. NO.	DRY WEIGHT OF LIVER TISSUE MG.	ORNITHINE CONC. MG. PER CENT	NH_4 CONC. MG. PER CENT	PERIOD OF OBSERVATION MINUTES	Q_{O_2}	Q_{urea}
12	34.2	0	0	111	12.52	0.29
13	19.6	0	0	110	13.68	0.14
16	18.0	6.5	0	92	9.96	0
17	28.0	6.5	0	90	10.79	0.18

In the course of the experiments with ornithine we observed that in the presence of this amino acid the rate of respiration of the liver slices was sometimes much less than in the parallel vessel not containing ornithine. This did not occur always; and was more frequent and more marked in lactate than in glucose Ringer's solution. In most of the experiments the rats used were from eighteen months to two years old; toward the end only one litter three months old was available. The inhibiting effect of ornithine was most marked on the livers of these animals. Typical results are given in table 4. They show that if ornithine is not added to the control vessel, its inhibitory influence on the total respiration may completely obscure an additional oxygen consumption in the experimental vessel.

The rates of urea synthesis observed by us are in most cases less than those reported by Krebs and Henseleit. This probably was because the carbon dioxide tension in our experiments was much lower, nearly zero, as compared with 5 per cent in the experiments of Krebs and Henseleit. This point is also under investigation.

The quotient Q_{O_2} is cubic millimeters of O_2 (reduced to standard temperature and pressure) used per hour, divided by the weight of the tissue in milligrams after drying at 100°C. Q_{urea} is similarly the number of cubic millimeters of CO_2 (reduced to standard temperature and pressure) obtained after the action of urease, divided by the duration of the urea synthesis in hours, and the dry weight of the tissue in milligrams.

Summary.—(1) A modification of the Warburg technique is described facilitating the handling and measurement of the respiration of a large number of tissue slices in one vessel.

(2) With this technique employing rat liver slices, an increased oxygen consumption was found to accompany the synthesis of urea from ammonium bicarbonate in Ringer's solution.

(3) It is pointed out that this process is probably responsible for part (not more than 20 per cent) of the specific dynamic action of protein.

[1] Krebs, H. A., and Henseleit, K., *Zeit physiol. Chem.*, **210**, 33 (1932).
[2] Warburg, O., *Biochem. Z.*, **142**, 317 (1923).
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ON THE INHERITANCE OF DIABETES MELLITUS

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Data have been collected on diabetes incidences in the families of 523 diabetic patients appearing at the Joslin Diabetic Unit from July, 1932, to October, 1932. A control group of 153 non-diabetic patients was similarly recorded. The data for diabetes incidences among parents and siblings of the patients in each group are given in table 1. Since diabetes incidence varies from decade to decade (see table 2, column 2), comparisons between the two groups are necessarily made by decades. It is obvious that the "diabetic" population contains a significant excess of diabetics among the parents and siblings. The statistical comparisons, using a 2×2 classification to test for independence for each decade, give a value of $\chi^2 = 18.366$, $P = 0.0095$ for the incidences among the parents and $\chi^2 = 22.153$, $P = 0.0047$ for the incidences among the siblings. Furthermore, 110 of the 523 patients or 22.94 per cent reported diabetic blood-relatives other than parents and siblings, whereas but 16 of the 153 non-diabetic patients, or 10.46 per cent, reported such diabetic relatives;

the difference is statistically significant on the basis of the crude percentage probably errors.

Examining the possible hereditary basis of this significant excess of diabetics in the families of the diabetic patients we adopt the simplest hypothesis consistent with the facts, namely: that the capacity for developing diabetes is inherited as a simple Mendelian recessive. We denote the gene governing this capacity by m, its dominant allelomorph by M. Since each mating in the diabetic group contains at least one diabetic offspring there can be only three crosses on this hypothesis:

TABLE 1*

COMPARATIVE INCIDENCE OF DIABETES IN PARENTS AND SIBLINGS OF A CONTROL AND A "DIABETIC" POPULATION

	"DIABETIC" POPULATION			CONTROL POPULATION			"DIABETIC" POPULATION			CONTROL POPULATION		
DECADE	DIABETIC PARENTS	NON-DIABETIC PARENTS	PER CENT DIABETIC	DIABETIC PARENTS	NON-DIABETIC PARENTS	PER CENT DIABETIC	DIABETIC SIBLINGS	NON-DIABETIC SIBLINGS	PER CENT DIABETIC	DIABETIC SIBLINGS	NON-DIABETIC SIBLINGS	PER CENT DIABETIC
1	..	..	...	.	..	..	4	243	1.46	0	90	0.00
2	..	..	...	.	..	..	7	141	4.73	1	90	1.11
3	0	16	0.00	0	6	0.00	3	216	1.37	0	120	0.00
4	4	119	3.25	0	14	0.00	7	207	3.27	1	104	0.96
5	6	134	4.29	0	54	0.00	11	259	4.07	0	94	0.00
6	20	153	11.56	1	76	1.39	26	319	6.96	0	77	0.00
7	32	194	14.16	2	62	3.23	32	224	12.50	2	44	4.55
8	18	180	9.09	3	56	5.35	10	92	9.80	0	23	0.00
9	5	121	3.97	0	29	0.00	0	8	0.00	0	5	0.00
10	1	29	8.33	0	3	0.00	..	..	...	.	..	..
Totals	86	946	8.33	6	300	1.96	100	1709	5.85	4	647	0.62

* Several parents whose ages were unknown have been omitted from this table.

(1) $Mm \times Mm$, (2) $Mm \times mm$ and (3) $mm \times mm$. We have examined in detail the crosses (1) and (2) as the conjugal diabetics are too few for extensive analysis. Assuming for the moment that all mm individuals among the parents have been identified as diabetics, all matings with neither parent diabetic have been classified as $Mm \times Mm$, and those with one parent diabetic as $Mm \times mm$.

Classifying the matings according to number of children per family, we calculate the expectation of mm individuals among the offspring as $\frac{p}{1-(q)^s}$, where p is the expectation of mm, q the expectation of in-

dividuals not *mm*, and *s* the number of children per family. Since the patients serve merely to bring a given family into our population they are omitted from subsequent calculations and the expected incidences of *mm* individuals among the siblings are employed for comparison with the observed incidences.

It is not to be expected that all *mm* individuals among the siblings have been identified, since a number of persons capable of developing diabetes have either died before it could be manifested, or have not yet attained an age or condition at which it would be manifested, and our only clue to *mm* individuals is the actual occurrence of diabetes. If we assume, however, that potential diabetics, *until the time that they develop*

TABLE 2

THE EXPECTED AND OBSERVED INCIDENCES OF DIABETICS IN THE SIBLINGS OF PATIENTS WITH BOTH PARENTS NON-DIABETIC (I.E., *Mm* × *Mm*)

DECADE	POTENTIAL DIABETICS EXPECTED	PER CENT IDENTIFIABLE (JOSLIN, U. S. A.)	NUMBER IDENTIFIABLE	NUMBER OBSERVED	TOTAL NUMBER OF SIBLINGS	NUMBER IDENTIFIABLE (ADAMS, U. S. A.)	NUMBER IDENTIFIABLE (THIS POPULATION U. S. A.)
1	34.74	2.69	0.93	3	209	0.88	1.83
2	21.59	4.06	0.88	6	134	0.82	1.84
3	32.41	5.54	1.80	1	196	2.12	2.38
4	28.15	10.16	2.86	6	168	2.76	2.47
5	35.44	23.22	8.23	7	210	6.91	7.18
6	46.81	42.36	19.83	14	281	19.26	18.90
7	33.19	56.13	18.63	20	201	23.31	19.59
8	14.31	71.26	10.20	7	88	10.97	9.17
9	1.32	100.00	1.32	0	8	1.32	1.32
Totals	247.96		64.68	64	1495	68.35	64.68
Deviation			0.68			4.35	0.68
Probable error			±5.31			±5.45	±5.31
Deviation / Probable error			0.13			0.80	0.13

diabetes, are subject to the same chances of death as are persons generally, then we can determine the proportion of observed diabetics to potential diabetics for each decade by employing the known age-incidence tables for diabetes and correcting these on the basis of the general life tables. Using Glover's 1910 tables for the United States and the age-incidence data of: (1) Dr. E. P. Joslin's first 6000 cases of diabetes, (2) Adams's cases from the Mayo Clinic and (3) our population of 523 diabetic patients, we have calculated the real expectation of diabetics per decade among the siblings in the two crosses (tables 2 and 3). The calculated percentages of diabetics are given in table 2 only for the data of Joslin; calculations based on the other two sets of data are not markedly different, as can be seen from the expectation figures.

The expected number of *mm* individuals per decade among the siblings has been determined by collecting the expectations for the various-sized families and assigning an expectation for each decade proportionate to the total number present in each decade.

The expected and observed totals agree remarkably well, regardless of the source of the incidence data. There is a slightly better agreement between observed and expected numbers per decade when the incidence data for the patients of this population are employed (last columns of tables 2 and 3). This is to be expected if there were a positive correlation

TABLE 3

THE EXPECTED AND OBSERVED INCIDENCES OF DIABETICS IN SIBLINGS OF PATIENTS WITH ONE DIABETIC PARENT (I.E., *Mm* × *mm*)

DECADE	POTENTIAL DIABETICS EXPECTED	NUMBER IDENTIFIABLE (JOSLIN)	NUMBER OBSERVED	TOTAL NUMBER SIBLINGS	NUMBER IDENTIFIABLE (ADAMS)	NUMBER IDENTIFIABLE (THIS POPULATION)
1	15.83	0.43	1	38	0.40	0.83
2	5.58	0.23	1	14	0.21	0.48
3	9.50	0.53	2	23	0.34	0.70
4	18.32	1.86	1	46	1.79	1.60
5	23.32	5.41	3	58	4.55	4.72
6	25.39	10.76	12	62	10.45	10.25
7	18.66	10.47	9	47	13.10	11.01
8	4.43	3.16	3	11	3.40	2.84
Totals	121.03	32.85	32	299	34.24	32.43
Deviation		0.85			2.24	0.43
Probable error		±3.65			±3.71	±3.63
Deviation / Probable error		0.23			0.60	0.12

among siblings for the age of onset of diabetes. It is scarcely feasible to employ the χ^2 test for the goodness of fit per decade with the relatively small numbers here available (cf. Wilson, Hilferty and Maher;[2] Warwick[3]). When, however, the probabilities of the observed ratios are calculated for each decade, it is found that only in the second decade is there a significant departure from expectation.

We may avoid the laborious calculation incident to the assembling of tables 2 and 3 by simply assuming that the hazards preventing the expression of *mm* (i.e., as diabetes) act equally upon the siblings of the two groups. This view is crudely defensible since the median age of the siblings in the matings listed as *Mm* × *Mm* is approximately 42 years, and in the matings *Mm* × *mm* 45 years. We then expect the ratio of diabetics in the two groups to be the same as the ratio of expected *mm* individuals. Actually we expect 43.82 per cent of the siblings to be *mm* in the cross *Mm* × *mm* and 16.59 per cent in the cross *Mm* × *Mm*. The

ratio is 1:2.64. We observe 10.70 per cent and 4.28 per cent of diabetics in these two crosses. The ratio is 1:2.50; the difference between these expected and observed ratios is less than the probable errors of the difference.

The probability exists that a number of the parents listed as *Mm* are really *mm*, but have not been identified as such. This would increase the expectation of *mm* individuals among the offspring and consequently the observed number of diabetics would be less than the expected number. How great this probable deficiency of diabetics would be depends on the number of *mm* parents unidentified. If these parents are offspring of a population breeding at random, and if the incidence of potential diabetics (*mm* individuals) is 4 per cent, then it can be demonstrated that there are unidentified 22 *mm* individuals among these parents. This would not significantly alter the expectation of diabetics among the offspring. If there were as many as 80 *mm* parents now listed as *Mm* the expected diabetes incidence among the siblings would be increased by at most 20 individuals.

Despite the obvious imperfections of a statistical analysis of the type of data here considered, we believe that the approach employed is the only possible one since we deal with a condition that is manifested with advancing age. An ideal genealogy would require that parents and children live into the 9th decade. Ordinary genealogies might therefore be taken to indicate a spuriously low ratio of diabetics to non-diabetics.

Summary.—There is a statistically significant excess of diabetic relatives in the families of a group of diabetic patients when comparison is made with the incidences in families of non-diabetic patients. These high incidences in "diabetic" families may be accounted for statistically if we assume that the capacity for developing diabetes is inherited as a simple Mendelian recessive, provided: (1) that potential diabetics before they develop diabetes have the same chances of death as persons generally, and (2) that we have identified at least half of the genetically diabetic individuals among the parents of the "diabetic" population examined.

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GROWTH-PROMOTING SUBSTANCE AND ILLUMINATION

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The presence of growth-promoting substance—*auxin* (Kögl and Haagen Smit, 1931)—in the tip of the coleoptile of *Avena* has been proved repeatedly by several investigators (Went, 1928; Dolk, 1931), through its effect on elongation of shoots of species related or not to the one used as source of supply. Most work has been done with etiolated seedlings, or at least with seedlings grown in the dark for the greater part of their active growth. This obviously must be done in order to prevent conflict with the phototropic reactions particularly marked in *Avena*, one of the most widely used species for this type of experiment.

In the course of experiments on the resistance of auxin to light, I have had occasion to compare the production of the substance in plants grown under the same conditions of temperature, moisture and substrate but differing as to the presence or absence of light. Brief indication of a possible favorable effect of light on the yield of growth-promoting substance from *Rhizopus suinus* is found in a recent paper by Thimann and Dolk (1933). Germination of *Lupinus albus* seeds was done on sawdust, after a 10 hours' soaking in sterile distilled water, in incubators at 25° ± 0.5°C. One of the incubators was lighted by one 150-watt daylight bulb placed about 35 cm. above the seed-bed; the other incubator was kept totally dark. Seedlings were used when the length of the hypocotyle was 3.5 to 4.0 cm. long. It should be noted that at this moment the "dark-grown" seedling does not yet show the typical elongated stem so characteristic of the etiolated seedlings, but is very similar in appearance to the "light-grown" seedling except in color of the cotyledons (yellow for the "dark-grown," green for the "light-grown").

Each seedling was cut 2 to 3 mm. below the point of insertion of the cotyledons and the apical part so removed from the seedling was placed in contact for 60 minutes with an agar block 2 × 2 mm. in cross-section and 0.8 to 1.0 mm. thick.

The estimation of the quantity of auxin liberated is made in the usual manner, the decapitated coleoptile of *Avena* being the test-object. For a more detailed description of the technique, cf. Dolk, 1931, and Navez, 1932. The outstanding result obtained, yet rather unexpectedly, was that there is liberated from the apex of the "light-grown" seedling about twice as much auxin as from the apical region of the etiolated seedling. The following figures, averages from series of ten seedlings each, express the angle of bending of standard coleoptiles of *Avena* which have been

asymmetrically provided with the diffusates from "light"- and "dark"-grown *Lupinus* tips, for 120 minutes.

"LIGHT-GROWN"		"DARK-GROWN"	
10.5°		3.9°	
9.0°	Mean, 10.4°	6.1°	Mean, 5.1°
11.9°		5.2°	

This finding is interesting as it may point to the fact that auxin is formed more abundantly in light than in dark. It may thus be linked directly or indirectly with photosynthesis, or with some product of photosynthesis. It is to be understood, of course, that the observations refer to the effect of extract of tip as prepared.

Plants grown in total darkness always stop growing before total exhaustion of the reserve materials stored in the cotyledons (reducing substances are still present in cotyledons). It might be conceivable that in our "light-grown" plants the small amount of light with which the seeds were illuminated when put to soak was enough to act as a trigger for the release of the mechanism which liberates a small amount of auxin at the start of germination and permits a more complete utilization of the reserve substances. It should be noted that the seeds of *Lupinus* used here, whether soaked and germinated in total darkness or irradiated during the soaking process by a 150-watt lamp, resemble one another very much, although one might state that the illuminated ones seem to germinate a little earlier (6 hours) than the dark-grown ones.

According to experiments performed by Went (1928) we know that in the intact coleoptile of *Avena* the amount of auxin extractable from the photically stimulated side of the organ is effectively decreased by side-illumination; in seeds stimulated from above, Went found also a notable decrease in the amount of auxin after stimulation. Here, on the contrary, illumination of a short duration of the *Lupinus* seeds during the early stages of germination decidedly increases the amount of auxin extractable. Nevertheless the two experiments are not necessarily contradictory, as the moment of irradiation is different in each case. At the same time, this result might have to be taken into account when one deals with tropistic responses involving auxin as one step in the mechanism of the reaction.

Summary.—Apical portions of seedlings of *Lupinus albus* grown under constant conditions in an illuminated thermostat release to agar blocks a larger amount of auxin than dark-grown seedlings. The relative amount seems to be of the order of 2 to 1, as measured by the angle of curvature induced in decapitated coleoptiles of *Avena*.

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THE EFFECT OF ICE AND STEAM WATER ON EUGLENA

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Previous papers (Barnes, 1932; Barnes and Barnes, 1932; Lloyd and Barnes, 1932) have indicated that there is some important difference in water that has recently been in the form of ice and water which has recently been in the form of steam as judged from its effect on the cells of *Spirogyra*. In this work the microscopical appearance of the cells was used as the indicator, for it is difficult to secure quantitative growth data on the filamentous algae. The present paper deals with the effect of these types of water on a protozoan, *Euglena gracilis*, in which it is possible to determine the number of cells per cc. of water. These experiments support the original findings that ice and steam water differ in some fundamental way, and they are not incompatible with the original working hypothesis that trihydrol particles are essential to certain types of cells.

The problem of the association of water is in a very unsettled condition as will appear in a review of the polymerization of water which we expect to publish soon. This will deal with all the important papers in the field from the original specific heat paper of Rowland (1879) in which it was shown that water recovers slowly from freezing to the more recent infrared analyses and the slow establishment of polymer equilibrium at low temperatures. Even if the original trihydrol hypothesis need be considerably modified as research proceeds, it is hoped that the work will stimulate further investigations concerning the properties of water. For example, one may cite the work of Menzies (1932) who compared the vapor pressure of samples of recently boiled and frozen water at the same temperature. The apparatus consisted of two 25-cc. Pyrex bulbs connected by a water manometer. Under these conditions Menzies found no appreciable difference in vapor pressure. It is doubtful, however, if vapor pressure is a reliable indicator of the concentration of water polymers. In fact, it is the only important property of water which previous investigators have not studied in relation to association. The vapor pressure of ice and water is treated in "Ice Engineering" by H. T. Barnes (pp. 31–33, 56–57). Barnes and Vipond in 1909 found the surprising result that the heat of vaporization of ice at 0°C. is almost identical with that

of water at 0°C. which indicates that ice evaporates as a polymeric vapor. In ordinary evaporization of ice the change of the solid vapor to ordinary vapor takes place just outside the surface and would escape detection in ordinary vapor pressure measurements. Moreover, in 1925 Washburn calculated the vapor pressure of ice and water at 0°C. on thermodynamical grounds based on the measurements of Weber. These calculations give the following vapor pressures: ice, 4.579; water, 4.579, both at 0°C.

For these reasons we think that other tests such as the rapidity of freezing might be more easily interpreted as indicative of the trihydrol concentration. It should also be noted that Menzies compares ice water with boiled water whereas we compare ice water and condensed steam water. We have developed a quantitative test showing the relative amount of trihydrol in ice and steam water at constant temperature (preparation of these types of water is described below). Ten cc. of each sample are placed in test tubes in Dewar flasks at −5°C. Graphs showing the freezing time at various temperatures will appear in a forthcoming review, but we may state at this time that 10 cc. of fresh ice water warmed to 10°C. will freeze in about ten minutes in a Dewar flask containing a brine mixture at −5°C. while condensed steam water cooled to 10°C. and placed under the same conditions will take over one-half hour for freezing. It should be pointed out that below −8°C. the samples freeze so rapidly that the difference in freezing time is apparently obliterated. Since the first feathery crystals form throughout the solution when the saturation point of trihydrol is exceeded, it is suggested that water containing more trihydrol will take less time to reach this point at moderately low temperatures.

In the experiments on Euglena all of the organisms were washed by centrifuging with water which had been distilled several weeks previously. The washed cells were then divided equally into two containers and fresh ice water was added to one half while fresh steam water was added to the other half. The steam water was prepared from a still entirely of Pyrex and the ice water was obtained from commercial block ice. Only perfectly clear pieces were used. These were thoroughly washed in distilled water and ice water. This ice (cf. Barnes and Lloyd, 1932) yields water of great purity if perfectly clear. Moreover, the crystals are much larger for they grow slowly with age (forthcoming review). Both kinds of water were at approximately the same temperature when added to the organisms. The initial concentration of organisms (in thousands per cc.) was determined (method described by Jahn, 1929) and the organisms were placed in centrifuge tubes in the light at a temperature of approximately 10°C. Under these conditions any slight difference in temperature between the types of water would rapidly disappear. Three tubes of each kind of water were used for each series; therefore each series was really an experi-

ment in triplicate. In seven of the eight series of experiments performed the water was changed once a day; in the eighth it was changed twice a day, and in this last series of experiments samples of the water used were subjected to the freezing tests described above. The average increase in the number of cells in the condensed steam water in these experiments (10–16 days' duration) was 32% (16.4 thousand to 21.7 thousand per cc.) whereas the average increase in the ice water was 105% (16.4 to 33.6 thousand per cc.) in the same time period. It is probable that gas equilibrium was the same in both types of water for pH determinations revealed no consistent difference which might be expected if the CO_2 content were appreciably greater in one type of water. Further details of these series will be published later.

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